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Population Genetic Structure of Bobcats (Lynx rufus) in South Dakota: Using Harvested Samples to Inform Management

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POPULATION GENETIC STRUCTURE OF BOBCATS (*Lynx rufus*) IN SOUTH DAKOTA: USING HARVESTED SAMPLES TO INFORM MANAGEMENT

BY

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THESIS ACCEPTANCE PAGE

Stuart C. Fetherston

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

POPULATION GENETIC STRUCTURE OF BOBCATS (*Lynx rufus*) IN SOUTH DAKOTA: USING HARVESTED SAMPLES TO INFORM MANAGEMENT

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A primary objective of state wildlife management agencies is to establish sustainable harvest levels for game species. An important component of sustainable management practices is the identification of appropriate management units for monitoring and establishing defensible harvest levels. Across their range, bobcats (*Lynx rufus*) are an ecologically and economically important species. Despite their importance, little is known about the genetic structure of bobcat populations in South Dakota. We used tissue sampled from n = 1,215 bobcats harvested across the state from 2014–2019 to infer population genetic structure. We used 17 microsatellite loci and a sex identification marker to assign individuals to genetically discrete clusters (i.e., populations) using Bayesian clustering algorithms. Analyses were run to identify the most likely number of clusters (K), considering potential values of K from 1 to 20. We found strong support for hierarchical structure at K = 2 and K = 4, as well as evidence of finer-scale structure that we were not able to fully evaluate due to the spatial resolution of the data. We calculated standard measures of population genetic diversity (e.g., heterozygosity) and population differentiation (e.g., F_{ST} and G''_{ST}). All pairwise measures of differentiation between identified clusters were found to be statistically significant ($P \leq 0.001$). We identified the spatial configuration of inferred clusters by geographically plotting individuals assigned to each cluster. The inferred structure reduced linkage disequilibrium and deviations from

Hardy-Weinberg Equilibrium as would be expected due to the Wahlund effect. For analyses supporting $K = 2$, the eastern and western clusters align closely with historical practices of managing bobcat harvest with 2 units in South Dakota, but our results suggest that shifting the boundary of the 2 units so the eastern unit includes counties immediately west of Missouri River and south of the Oahe Dam would better align management units with population boundaries. Alternatively, analyses supporting $K = 4$ provides a level of resolution that may benefit bobcat management if managers aim to conserve the uniqueness of bobcats in different regions (e.g., Black Hills).

INTRODUCTION

A primary requirement of wildlife managers when establishing sustainable harvest is the identification of biologically appropriate wildlife management units. Using large management units encompassing multiple discrete populations could lead to overharvest of some populations, even if overall harvest is low (e.g., if harvest within a management unit was not distributed evenly or was concentrated on a specific population; Taylor and Dizon 1999). In contrast, small management units may not sufficiently encompass the area required to support a population (Rosenberry and Diefenbach 2019) and may lead to increased management costs (Allendorf et al. 2013). Therefore, it has been recommended that management units be delineated through the identification of demographically independent populations with population dynamics that depend more on local reproduction and mortality than metapopulation dynamics (Palsbøll et al. 2007).

Delineating management units for harvested species can be accomplished using several approaches. Management units have commonly been defined based on geographical features (e.g., watersheds), political boundaries, or other features that are easily identified by hunters or trappers (Connelly et al. 2012), but this approach may not consider population units and could lead to a mismatch between the scale of populations and scale of management (Conner and Miller 2004). Establishing management units based on demographically independent populations requires information on connectivity or rates of movement among populations, which is commonly assessed through techniques such as telemetry (Amstrup et al. 2004, Viengkone et al. 2018) or modeling of habitat suitability and connectivity (Dickson et al. 2013). Telemetry is useful in collecting data related to dispersal, but dispersal does not necessarily lead to gene flow and may be misleading if

used to infer independent populations (Koenig et al. 1996, Moore et al. 2017). Similarly, modeling of habitat suitability often relies on expert opinion or presence data that does not necessarily reflect gene flow; when tested against landscape permeability models informed by genetic data, habitat suitability models are not an accurate surrogate for landscape connectivity and as such may also be misleading when inferring independent populations (Mateo-Sánchez et al. 2015).

Alternatively, management units based on demographically independent populations may be identified using genetics (Moritz 1994). Population genetic structure is defined as the nonrandom distribution of genotypes in space and time and develops due to environmental features or biological characteristics of the species that influence dispersal patterns (Nunney 2001). Landscape heterogeneity influences the genetic structure of populations through isolation effects that can differentiate populations due to geographic distances (Wright 1942), absolute or permeable barriers (McRae 2006), or stark changes between environments (Wang and Bradburd 2014). Genetic structure may also be influenced by the physical and physiological ability of a species to disperse (Hillman et al. 2014) and behavioral differences among individuals based on age, reproductive status, or sex (Tiedemann et al. 2000).

A commonly used genetic definition of management units was provided by Moritz (1994), who described management units as populations that differed significantly in allele frequencies, regardless of their phylogenetic distinctiveness. Determining populations in this manner allows for fine-scale management and monitoring of discrete populations, which is essential to identifying potentially vulnerable populations, establishing sustainable harvest management, or both. When evaluating the genetic

structure of continuously distributed carnivores, we may expect patterns of panmixia (i.e., no spatial structure) or isolation by distance due to their large home ranges and high dispersal capacity (Sunquist and Sunquist 2001). Despite this, many studies on carnivore populations have found patterns of discrete genetic structure associated with anthropogenic barriers (e.g., roads; Riley et al. 2006), reduced landscape permeability due to unfavorable land-cover types (McRae et al. 2005) or natural barriers (e.g., rivers; Trizio et al. 2005), or macro- (Sacks et al. 2004) or micro- (Lonsinger et al. 2015) environmental changes in the absence of substantial barriers or distances between populations.

Bobcats (*Lynx rufus*; Schreber 1777) are the most widely distributed felid in North America (Anderson and Lovallo 2003). A broad-scale assessment of bobcat population trends found that populations were stable or increasing and were not severely fragmented throughout the contiguous United States (Roberts and Crimmins 2010). Although the International Union for Conservation of Nature (IUCN) classifies bobcats as a species of "least concern" (Kelly et al. 2016), the Convention of the International Trade in Endangered Species of Wild Fauna and Flora (CITES) still regulates international trade of bobcats under Appendix II. As an Appendix II species, bobcats are regulated due to similarity in appearance to species classified under Appendix I for conservation concern (CITES 2020). In the United States, regulated harvest is permitted in 40 of the 47 states where bobcats occur. Harvest may lead to additive mortality for bobcats (Anderson and Lovallo 2003), and therefore, it is important for managers to identify appropriate management units for monitoring populations and regulating harvest. Applying population genetic principles can provide managers with defensible identification of

demographically independent populations for use as management units (Reding et al. 2013).

In the midwestern United States, bobcats were extirpated from much of their historical range following European colonization and did not begin to recolonize the region until the late 20th century; bobcats at that time were still uncommon, and the need for protection and management was recognized (Koehler 1987). South Dakota established a statewide harvest season in 1975. In the 1977–1978 season, harvest was restricted to counties west of the Missouri River. South Dakota counties east of the Missouri River were closed to harvest for 35 years until limited harvest in 5 counties was initiated in 2012 (Broecher 2012), followed by 5 additional counties in 2018 (SDGFP 2018), and all remaining counties in 2021 (SDGFP 2021).

Bobcats are highly mobile and panmictic in some regions (Croteau et al. 2010, Reid 2006), but there is evidence of fine-scale population genetic structure in other portions of their range (Reding et al. 2013, Smith et al. 2020, Reed et al. 2017). Large-scale regional assessment of bobcat population genetic structure in the Midwest did not include samples from South Dakota that originated from east of the Missouri River (Reding 2011), and no studies have focused on the fine-scale genetic structure of bobcats in South Dakota. We used genetic samples from harvested bobcats to inform wildlife management in South Dakota. Our first objective was to test for genetic structure in bobcats in the state against a null hypothesis of panmixia. We predicted that there would be genetic structure in the population due to changes in the environment across the landscape. Notably, we predicted that bobcats in the Black Hills region would be a discrete population due to the distinctiveness of the habitat in the Black Hills when compared to the rest of the state.

We also predicted that prominent linear features on the landscape, including a large highway (i.e., Interstate 90) and large rivers (e.g., Missouri River), would constitute barriers to bobcat movement and that bobcat populations would be demarcated by these features. Our second objective was to evaluate whether the population genetics of bobcats supported the current harvest units used by wildlife managers (i.e., east versus west of the Missouri River).

STUDY AREA

The study area included counties in South Dakota with bobcat harvest between December 2014 and February 2019, as well as 1 county (Minnehaha County) where South Dakota Game, Fish and Parks (SDGFP) officials collected a non-harvest bobcat sample (Figure 1). During the period of sample collection, bobcat harvest in the state was divided into 3 management zones. In the 22 counties west of the Missouri River (hereafter, West River), unrestricted harvest (i.e., no bag limit) was permitted from late December through mid-February. A more limited harvest (i.e., bag limit = 1) was permitted in 10 counties east of the Missouri River (hereafter, East River) over a shorter harvest season from late December through mid-January. Harvest was not permitted in the remaining 34 counties east of the Missouri River (Figure 1; SDGFP 2020).

Precipitation varied across South Dakota. Eastern South Dakota had a dry subhumid climate and received an annual mean precipitation of 63 cm (range = 37-98 cm) from 2014–2019. Western South Dakota had a semi-arid climate and received an annual mean precipitation of 55 cm (range = 29–85 cm) from 2014–2019 (NOAA 2021). Compared to eastern South Dakota, the lower precipitation and higher evaporation produced a larger

moisture deficit in western South Dakota. The Black Hills region is a notable outlier in western South Dakota, where montane topography and land cover reduce evaporation (Widrlechner 1999). South Dakota experiences considerable temporal (e.g., seasonal) variability in precipitation resulting in flooding and moderate to severe droughts, but there is evidence that bobcat populations are resilient to drought conditions (Watts 2015, NDMC 2021).

Located in the Northern Great Plains, South Dakota is broadly characterized as a Great Plains ecosystem (Omernik 2004). The only markedly different region is the Black Hills, representing an eastern extension of the Rocky Mountain. South Dakota consists of 8 level-III ecoregions (EPA 2013). The eastern third of South Dakota is predominantly Northern Glaciated Plains (28.5%), with marginal amounts of Western Corn Belt Plains (1.9%) and Lake Agassiz Plains (0.1%) in the southeastern and northeastern corners of the state, respectively. Northwestern Glaciated Plains (15.9%) span central South Dakota from north to south, predominantly east of the Missouri River. Western South Dakota is predominantly Northwestern Great Plains (47.8%), with Middle Rockies (4.0%, i.e., the Black Hills) in the west and marginal amounts of High Plains (1.2%) and Nebraska Sand Hills (0.6%) in the south. Eastern South Dakota has experienced a higher rate of land-use conversion (from native grasslands to row crop production, e.g., corn, *Zea mays*, and soybean, *Glycine max*) than in western South Dakota. Land-use conversion has been identified as an area of concern regarding the maintenance of native grassland ecosystems (Wright and Wimberly 2013). Expansion of row crops has replaced native grasslands as demands for commodity crops have increased. From 2006–2011, the greatest net grassland conversion to corn or soybean during that period was in South Dakota (a net

loss of 182,000 ha of grassland), and this was likely an underestimate given 'grassland' included pasture, hay, and fallow or idle fields, in addition to native grasslands (Wright and Wimberly 2013).

METHODS

Sampling

South Dakota Game, Fish and Parks officials collected tissue samples from the jaws of harvested bobcats presented for CITES export tags from 2014–2019. The samples were individually stored with a silica desiccant at room temperature prior to DNA extraction. The county of harvest was documented for each sample. For counties in southwestern South Dakota, whether the bobcat was harvested in the Black Hills or not was also documented. Information regarding harvest in the Black Hills allowed us to distinguish "subcounties", which are the portions of southwestern counties that included, or did not include, the Black Hills. We also obtained 1 sample from an incidentally trapped bobcat from a county that was closed to bobcat harvest.

Laboratory

We processed samples at the Laboratory for Ecological, Evolutionary and Conservation Genetics at the University of Idaho. We extracted samples using the QIAGEN DNeasy blood & tissue kit (Qiagen, Valencia, CA), and each extraction included a negative control to monitor for contamination. We initially extracted and amplified samples to perform a molecular sex identification test (Pilgrim et al. 2005). The DNA extract was archived and stored frozen at -80° C. We subsequently used the archived DNA to genotype samples using primers for nuclear DNA (nDNA) microsatellite loci to

investigate the genetic diversity and population genetic structure of bobcats. We screened 26 potential microsatellite loci (Table S1) for successful amplification on a subset of 15 samples. We excluded 9 microsatellite loci that failed to amplify, amplified weakly, showed evidence of null alleles, or had other irregularities (e.g., non-specific peaks) from further consideration. Of the remaining 17 microsatellite loci, 10 were from an established microsatellite multiplex for cougars (*Puma concolor*), which also included a sex marker and was able to be used unaltered (Table 1). The remaining 7 microsatellite loci made up a second bobcat multiplex specific to our project (Table 1). We ran polymerase chain reactions (PCR) utilizing a multi-tubes approach for reliable genotyping (Taberlet et al. 1996). Each PCR was set up for 7μL total volume using recommended protocols for Qiagen Multiplex PCR Kit including Q-solution (0.70 μL), Master Mix (3.50 μL), forward and reverse primers (10μM for each locus; volumes in Table 1), 1 μL of nDNA extract, and RNase-free water to make up the final volume. We performed PCR procedures on a Bio-Rad C1000 Touch 96 Well PCR Thermal Cycler (Bio-Rad, Hercules, CA) with optimized thermal profiles for each multiplex (Table 2). We combined LIZ 500 size standard (0.15 μL; Applied Biosystems, Inc., Foster City, CA) and 10 μL formamide with 1 μL of the PCR product. The PCR products were visualized with an ABI 3130xl (Applied Biosystems, Inc., Foster City, CA).

GeneMapper Software 6 (Thermo Fisher Scientific, Waltham, MA) was used to score alleles. The goal for each sample was to achieve consensus genotypes, consensus was reached when observing the same genotype at a particular locus across multiple replicates of the sample (Broquet and Petit 2004). A consensus genotype required at least 2 observations of the genotype, whether homozygous or heterozygous. While it is more

common to require ≥2 consensus genotypes for heterozygotes and ≥3 for homozygotes (Lonsinger and Waits 2015), this is usually necessary because of the poorer quality and limited amount of DNA collected through noninvasive genetic sampling (usually of hair or feces). Given our use of higher quality tissue samples, observing a genotype twice was sufficient to form a consensus. Amplification was performed in duplicate to minimize the influence of genotyping errors, and if amplification failed to produce a consensus genotype after scoring the initial replicates, then up to 2 more additional replicates were performed. We used program R and package ConGenR to establish consensus genotypes and calculate genotyping error rates (Lonsinger and Waits 2015, R Core Team 2020).

Population Genetic Structure

The sample size and number of loci analyzed can both have a strong influence on the power to correctly identify genetic patterns (Landguth et al. 2012). To determine the minimum number of loci over which complete consensus genotypes were required for a sample to be included in the genetic structure analyses, we used GenAlEx v6.503 to calculate the probability of identity among siblings ($P_{(ID)sib}$) (Waits et al. 2001; Peakall and Smouse 2006, 2012). We used $P_{(ID)sib}$ instead of the probability of identity ($P_{(ID)}$) because $P_{(ID)}$ assumes the population does not contain closely related individuals or substructure. When these assumptions are not met, the number of loci necessary to differentiate individuals may be underestimated with $P_{(ID)}$ (Waits et al. 2001). In contrast, $P_{(ID)sib}$ gives a more conservative estimate of the probability of identification (Waits et al. 2001). Inclusion of closely related individuals in population clustering analyses may lead to biased inferences indicating support for structure when none exists (Anderson and Dunham 2008). We used GenAlEx to estimate relatedness (*r*) using the Queller and

Goodnight (1989) method, which is robust with multilocus testing and does not require random mating in the population (Van de Casteele et al. 2001). We parsimoniously removed individuals that shared pairwise relatedness values $r \geq 0.45$ (first-order relatives; i.e., parent-offspring or full siblings) (Viricel and Rosel 2014).

We evaluated population genetic structure using 2 Bayesian clustering methods implemented in the programs Structure v2.3.4 (hereafter, STRUCTURE; Pritchard et al. 2000) and Bayesian Analysis of Population Structure v6.0 (hereafter, BAPS; Corander et al. 2006, Corander et al. 2008a, Cheng et al. 2011). STRUCTURE and BAPS evaluate genetic structure by identifying the most likely number of populations (*K*), which is determined by grouping individuals into genetic clusters that minimize deviations from Hardy-Weinberg equilibrium and reduce evidence of linkage disequilibrium. We used preliminary runs of STRUCTURE to assess stabilization of α (the Dirichlet parameter) and identify an appropriate number of burn-in replicates. These runs also were used to plot the log-likelihood of each *K* (L[*K*]) and observe an asymptote, which informed the maximum value of *K* to test. The range of *K* must be large enough to capture the true value of *K*. Based on these preliminary runs, subsequent analyses considered $K = 1–20$ and included 250,000 burn-ins and 500,000 Markov Chain Monte Carlo repetitions per run using the admixture and correlated allele models (Falush et al. 2003). We initially ran an aspatial analysis in STRUCTURE for 10 independent batches at each value of *K*. We then repeated the STRUCTURE analysis in a spatially implicit formulation using dummy variables to code samples into *a priori* spatial groups by sampling county or subcounty. Spatially implicit STRUCTURE models that incorporate sampling area information are better suited to detect weak patterns of genetic structure but ignore sampling information

when the structure is unrelated to the sampling area (Hubisz et al. 2009). To interpret the STRUCTURE results, we calculated the mean L(*K*) and delta *K* (Δ*K*) for each *K*. Delta *K* is the rate of change in the mean L(*K*) of each successive *K*, is commonly used to interpret STRUCTURE results, and, in most cases, correctly estimates the value of *K* (Evanno et al. 2005). We used Structure Harvester (Earl and von Holdt 2012) to plot the mean L(*K*) estimates and Δ*K* at each *K* for both STRUCTURE analyses.

We then investigated the genetic structure of bobcats using BAPS, which returns the most-supported value of *K* and eliminates the need to interpret potentially ambiguous summary statistics. Aligning with the range of *K* considered in our STRUCTURE analyses, we set the maximum *K* to 20. We first ran BAPS with the 'Clustering of Individuals' model, which was similar to the spatially implicit analysis in STRUCTURE and included the same samples and sampling information (i.e., spatial groupings). We also ran BAPS using the 'Spatial Clustering of Individuals' model, which can consider spatial coordinates in a spatially explicit analysis (Corander et al. 2008b, Cheng et al. 2013). Although unique spatial coordinates were not available for each sample, we generated unique spatial coordinates for each sample following Reding et al. (2013). Specifically, we identified centroids for each spatial group using the mean center processing tool in ArcPro v2.4.0 (Esri, Redlands, CA) and generated a random point for each sample within 250 m of the sample's group centroid. We used these spatial coordinates for each sample to perform the spatially explicit analysis with BAPS. For each BAPS analysis, we used 80 independent batches. BAPS stored results from each run and returned the most likely value of *K* as indicated by the maximum log-likelihood L(*K*).

To better discern patterns of agreement or disagreement among the 2 STRUCTURE and 2 BAPS analyses, we mapped the results of each analysis by classifying each spatial group by the genetic cluster to which the majority of the individuals sampled in that region were assigned. In addition to identifying the most prominent cluster for each spatial group, we also created pie charts depicting the proportion of individuals from each spatial group that were assigned to each genetic cluster. STRUCTURE results also included ancestry values (q) for each individual from the admixture model, which estimated the proportion of ancestry that was attributable to each genetic cluster. The ancestry values were used to evaluate the support for genetic clusters. If a cluster did not include any individuals for which $q \geq 0.70$, then it may have been over split or highly admixed. An over split cluster is one where the clustering analysis has inferred more clusters than exists, the over split cluster lacks a core area of occurrence, and the majority of individuals have ancestry that is split approximately evenly among clusters. In contrast to over split clusters, a cluster with high levels of gene flow, leading to high degrees of admixture, may also have few individuals with $q \geq 0.70$, but would still have a core area of occurrence.

GenAlEx was used to calculate differentiation statistics (F_{ST} and G''_{ST}), as well as associated *P*-values between pairs of clusters inferred by each Bayesian clustering analysis (Wright 1965, Meirmans and Hedrick 2011). F_{ST} is the most common of the *F*-statistics and is used to measure variance among populations and the loss of heterozygosity in populations (Wright 1965, Jost 2008, Whitlock 2011). G_{ST} was developed for use with loci having ≥2 alleles and many loci within and among populations. However, G_{ST} has issues when heterozygosity is high within populations, at which point the maximum range of G_{ST} falls below one, even when two populations share

no alleles (Nei 1973, Hedrick 2005). Hedrick developed G'_{ST} as a standardization of $G_{ST,}$ which divides G_{ST} by the maximum possible G_{ST} to ensure a range of 0–1 and allows for comparisons between loci with differing levels of variation, as is common for microsatellites (Hedrick 2005). Despite the improvements, G'_{ST} may be biased when dealing with a low number of populations (K), especially for pairwise comparisons. Consequently, G''_{ST} was developed to correct issues with sampling bias present in G'_{ST} (Meirmans and Hedrick 2011). Taking this into consideration, we quantified population differentiation between populations with both F_{ST} and G''_{ST}, as F_{ST} has historical precedence in the literature and G''_{ST} offers improvements in inference given our data (Whitlock 2011).

Genetic Diversity

We calculated genetic diversity metrics at each of the 17 loci in the complete sample and each cluster inferred by our Bayesian clustering programs unless otherwise noted. To account for multiple comparisons, we used sequential Bonferroni corrections in all evaluations of significance (Rice 1989). We used the genepop package in Program R (Raymond and Rousset 1995, R Core Team 2020) to calculate pairwise linkage disequilibrium (LD), Hardy-Weinberg equilibrium (HWE), and Weir and Cockerham's (1984) inbreeding coefficient (F_{IS}), which determined the extent to which deviations from HWE were positive or negative. Evidence of LD or deviations from HWE at loci in the total sample may indicate genetic structure due to the Wahlund effect (Allendorf et al. 2013). We used Fstat v2.9.4 (Goudet 2001) to calculate the number of alleles (A_N) and allelic richness (A_R), where allelic richness was a measure of the number of alleles at each locus corrected for sample size. Finally, we used GenAlEx to calculate the observed

and unbiased expected heterozygosity (H_O and H_E, respectively), which was the proportion of heterozygotes observed in the sample and the proportion of heterozygotes expected in a panmictic population. We calculated H_O and H_E at each locus for the total sample and for each inferred cluster.

RESULTS

We obtained tissue samples from 1,215 bobcats from 2014–2019 (n_{2014} = 13, n_{2015} = 4, n_{2016} = 223, n_{2017} = 209, n_{2018} = 449, n_{2019} = 317; Figure 1). Samples were collected across 31 counties, with a mean county-level sample size of 37.84 (SD = 37.96). Sample collection was not equal among regions, with more samples collected in West River (n = 1,102; county-level mean = 50.09, SD = 38.60) than East River (n = 71; county-level mean = 7.89, SD = 8.34). Our initial sample was 53.6% male and 43.5% female, with the sex for 2.9% of samples being unknown.

All samples were successfully extracted, and every sample amplified at ≥1 locus. The expected number of individuals with the same multilocus genotype was < 1 at $P_{(ID)sib}$ for 10 loci ($P_{(ID)sib}$ = 0.003%; expected number of individuals in the sample with the same multilocus genotype = 0.039). We removed 22 samples for which we could not establish complete consensus genotypes at ≥10 loci. We found 0.0016% of pairs had r > 0.45 and we removed 315 individuals . This was done parsimoniously by removing the individuals which occurred in the most pairs until all pairs had at least one individual removed. Following these reductions, we retained 878 samples (52.7% male, 45.3% female) from 31 counties (county-level mean = 27.55, SD = 27.89). Finally, we removed 23 samples

without sufficient location data from consideration for the Bayesian clustering analyses where the sampling area was taken into account.

The number of alleles across loci ranged from 7–19 (mean = 10.9, SD = 3.6; Table 3) and allelic richness was nearly identical, ranging from 7–19 (mean = 10.8, SD = 3.6; Table 3). In the total finalized sample, there was evidence of LD in 5.9% (8 of 136) of pairwise comparisons among loci and in 4.4% among samples with spatial data. In the total sample, we found significant departures from HWE in 10 of 17 loci, compared to 9 of 17 loci among only those samples with spatial data (Table 3). When considering the total sample, values of F_{IS} were positive and $H_O < H_E$ for all loci, indicating a heterozygote deficiency (Table 3). Mean H_O was 0.735 (SD = 0.104, range = 0.84–0.46) for the total sample and 0.734 (SD = 0.102, range = 0.84–0.47) for the total sample with spatial data (Table 3).

All 4 analyses with the 2 Bayesian clustering programs found support for genetic structure in the population. The aspatial STRUCTURE analysis provided support at $K = 2$ and $K = 12$ based on peaks in ΔK (Figure 2a); results based on the $L(K)$ were less clear, but estimates began to plateau at $K = 12$ with uncertainty (confidence intervals) increasing for higher K values (Figure 2b). Based on ΔK, the spatially implicit STRUCTURE analysis found support for $K = 2$, $K = 4$, and $K = 10$ (Figure 2c). Support based on $L(K)$ was again more ambiguous (Figure 2d). Results from the BAPS analyses agreed with those of STRUCTURE. The spatially implicit BAPS analysis found $K = 10$ to be most likely, whereas the spatially explicit BAPS analysis indicated support for $K = 2$.

For all 3 analyses that found support for $K = 2$, the divide between the eastern and western clusters was in the southcentral region of the state and west of the Missouri

River. Visually plotting the results for $K = 2$ showed geographic congruence for 2 clusters among the different analyses, representing an eastern and a western cluster (Figure 3). The notable outlier in the spatially explicit BAPS analysis was a single county (Yankton) in the southeastern corner of the state, in which a higher proportion of individuals were assigned to the western cluster. Genetic clusters were significantly different from one another across all 4 analyses ($P < 0.001$). In all 3 analyses that found support for $K = 2$, measures of F_{ST} and G''_{ST} suggested the eastern and western clusters were significantly different from one another. Differentiation as measured by G''_{ST} suggested moderate levels of differentiation (between 0.05–0.15; Wright 1978), with estimated differentiation being lowest for the aspatial STRUCTURE analysis compared to the spatially implicit STRUCTURE and spatially explicit BAPS analyses (Table 4). For $K = 4$ results supported by the spatially implicit STRUCTURE analysis, F_{ST} and G''_{ST} suggested significant differentiation between all 4 inferred clusters. There was moderate differentiation among the western clusters (i.e., northwestern, southcentral, and Black Hills) and moderate to strong differentiation between the eastern cluster and each of the 3 western clusters. (Table 5). For results of analyses with $K > 4$, F_{ST} and G''_{ST} suggested significant differentiation between all inferred clusters. In the aspatial STRUCTURE analysis, the clusters that nested into the eastern and western clusters at $K = 2$ of the same analysis were more different between one another than the eastern or western clusters were among themselves (Table 6). The spatially implicit STRUCTURE analysis showed a similar pattern with eastern clusters from $K = 2$ of the same analysis being more different from western clusters than they were from one another (Table 7). The spatially implicit BAPS analysis also suggested significant differentiation between all genetic clusters, but

1 cluster was excluded from analysis of F_{ST} and G''_{ST} due to a small sample size (Table 8).

For the aspatial STRUCTURE analysis, >60% of sampled individuals in both the eastern and western clusters were not substantially admixed ($q \geq 0.70$) (Table 9). Most individuals in the spatially implicit STRUCTURE analysis were also not substantially admixed (eastern cluster = 78.8%, western cluster = 87%; Table 10). There was a consistent pattern across the 3 analyses supporting $K = 2$, where counties nearest to the geographical divide between the eastern and western clusters had proportionally more individuals assigning to each cluster, compared to the counties further from the divide, where most individuals strongly assigned to either the eastern or western cluster.

The partition of samples into $K = 2$ reduced deviations from HWE and instances of LD compared to the total sample. For the inferred clusters from the aspatial STRUCTURE analysis, there was evidence of LD in 2.2% of comparisons in the eastern cluster and 2.9% in the western cluster (3 and 4 pairwise comparisons among loci out of 136, respectively). Six of 17 loci deviated from HWE in both the eastern and western clusters (Table 11). The mean allelic richness in the eastern cluster was 10.1 (SD = 3.4) and 10.0 (SD = 2.8) in the western cluster (Table 11). The aspatial STRUCTURE mean H_O was 0.722 (SD = 0.107, range = 0.85–0.44) for the eastern cluster and 0.745 (SD = 0.104, range = 0.83–0.47) for the western cluster (Table 11). For the inferred clusters from the spatially implicit STRUCTURE and those from the spatially explicit BAPS analyses, deviations from HWE and LD were lessened in comparison to the total sample and to a greater degree for the eastern cluster than the western cluster. In the inferred clusters from the spatially implicit STRUCTURE analysis, there was evidence of LD in 0.7% of comparisons (1 of 136

pairwise comparisons among loci) in the eastern cluster and in 2.2% of comparisons (3 of 136 pairwise comparisons among loci) in the western cluster. Results for HWE were similar, with 4 and 7 (of 17) loci deviating significantly from HWE in the eastern and western clusters, respectively (Table 12). The mean allelic richness in the eastern cluster was 9.8 (SD = 3.2) and 9.7 (SD = 2.6) in the western cluster (Table 12). The spatially implicit STRUCTURE mean H_O was 0.724 (SD = 0.105, range = 0.85–0.48) for the eastern cluster and 0.739 (SD = 0.104, range = 0.84–0.46) for the western cluster (Table 12). We did not find evidence of LD for the eastern cluster inferred by the spatially explicit BAPS analysis, but we did find evidence of LD for 1.5% (2 of 136) of pairwise comparisons among loci in the western cluster. Significant deviations from HWE occurred in 5 and in 7 loci in the eastern and western clusters, respectively (Table 13). The mean allelic richness in the eastern cluster was 9.3 (SD = 3.1) and 9.7 (SD = 2.6) in the western cluster (Table 13). The spatially explicit BAPS mean H_O was 0.721 (SD = 0.104, range = 0.84–0.48) for the eastern cluster and 0.739 (SD = 0.104, range = 0.84 – 0.46) for the western cluster (Table 13).

When mapped, results of the spatially implicit STRUCTURE analysis for $K = 4$ identified 1 cluster that aligned with the same counties as the eastern cluster identified at $K = 2$ of the same analysis. The remaining 3 clusters were nested within the western cluster identified by the spatially implicit STRUCTURE results for $K = 2$ and aligned with the northwestern, southcentral, and Black Hills regions of South Dakota (Figure 3d). Greater than half of individuals were not substantially admixed for 3 of the 4 inferred clusters, while in the southcentral cluster only 19.2% of samples were not substantially admixed (range 19.2–67.8%; Table 10). Instances of LD were reduced for each cluster compared to the total

sample, with the further division of the western cluster reducing LD more than the relatively unchanged eastern cluster, which had more instances of LD than the eastern cluster of the spatially implicit K =2. In the eastern cluster, there was significant evidence of LD in 2.9% of comparisons (4 of 136 pairwise comparisons among loci) and 1.5% of comparisons (2 of 136 pairwise loci comparisons) in the northwestern cluster. There were no instances of LD in the southcentral or Black Hills clusters. The eastern cluster remained relatively unchanged from the K = 2 eastern cluster in terms of HWE, with 4 of the 17 loci significantly deviating. The division of the western cluster into the northwestern, southcentral, and Black Hills clusters led to fewer loci deviating from HWE in all 3 clusters, with significant deviations from HWE in 4 of 17 loci, 2 of 17 loci, and 3 of 17 loci, respectively (Table 14). The mean allelic richness was 9.2 (SD = 2.9) in the eastern cluster, 8.8 (SD = 2.2) in the northwestern cluster, 8.9 (SD = 2.3) in the southcentral cluster, and 9.2 (SD = 2.6) in the Black Hills cluster (Table 14). The mean H_O for eastern, northwestern, southcentral, and Black Hills was 0.722 (SD = 0.100, range = 0.83–0.49), 0.726 (SD = 0.107, range = 0.85–0.46), 0.743 (SD = 0.108, range = 0.84–0.48), and 0.752 (SD = 0.112, range = 0.88–0.45), respectively (Table 14).

Finally, there was some evidence of fine-scale, hierarchical structure beyond K = 4 from the aspatial STRUCTURE, spatially implicit STRUCTURE, and spatially implicit BAPS analyses. The aspatial STRUCTURE analysis provided support for K = 12. When mapped, some of the clusters were geographically disjointed. There remained a pattern of eastern and western groupings, where 5 of the clusters inferred at K = 12 were nested within the eastern cluster of K = 2, 6 were nested within the western cluster, and 1 cluster plotted over both (Figure 4a). No clusters had >50% of individuals with q ancestry values >0.70

for the assigned cluster (range: 17.2% - 45.3%; Table 9). The spatially implicit analyses for both STRUCTURE and BAPS found support for $K = 10$. When plotted, the spatially implicit STRUCTURE $K = 10$ inferred clusters that were geographically disjointed (Figure 4b), and no clusters had >50% of individuals assigned with $q > 0.70$ (range 8.1% - 44.4%; Table 10). The spatially implicit BAPS analysis with support for $K = 10$ also revealed geographically disjointed county assignments and contained clusters with very few individual assignments; thus, these clusters were not represented as most prominent in any counties or subcounties (Figure 4c).

The partitions of $K = 12$ and $K = 10$ reduced HWE and LD compared to the total samples. In the aspatial STRUCTURE analysis for $K = 12$, 4 clusters showed evidence of LD in 1 of 136 pairwise comparisons among loci and all inferred clusters showed fewer departures (1–3 loci) from HWE than the total sample (Table S2). For each of the clusters, mean H_O ranged from 0.699–0.770 (Table 15). In the spatially implicit STRUCTURE analysis for $K = 10$, 7 clusters showed significant evidence of LD; 3 clusters had evidence of LD in 1 of 136 pairwise comparisons among loci, whereas 4 clusters had evidence of LD in 2 of 136 comparisons. In the spatially implicit STRUCTURE analysis, 8 inferred clusters had ≥1 departure from HWE (Table S3). For each of the clusters, mean H_O ranged from 0.703–0.755 (Table 15). In the spatially implicit BAPS analysis for $K = 10$, we found significant evidence of LD in 1 of 136 pairwise comparisons among loci for 1 cluster, and 2 of 136 comparisons for 3 clusters. The spatially implicit BAPS analysis found that 7 clusters had significant departures from HWE at 1–4 of the loci, with all departures suggesting a heterozygote deficiency (Table S4). For each of the clusters, mean H_O ranged from 0.700 – 0.802 (Table 15).

DISCUSSION

Based on our results, the most appropriate number of management units for bobcats in South Dakota is likely 2 or 4. The number of populations with support across the greatest number of analyses was $K = 2$. The eastern and western clusters align closely with historical practices of managing bobcat harvest with 2 units, but our results suggest that shifting the boundary of the 2 units so the eastern unit includes counties immediately west of Missouri River and south of the Oahe Dam would better align management units with population boundaries. Because there is evidence of finer genetic structure in the state, 2 management units may be too large if one or both units contain multiple discrete populations, which could lead to overharvest of unique populations being managed as part of a larger area (Taylor and Dizon 1999). We may have observed more deviations from HWE for $K = 2$ than $K = 4$ due to a Wahlund effect, suggesting there was cryptic structure that was not being accounted for in analyses for $K = 2$. Additionally, $K = 4$ captured a pattern of bobcat population genetic structure that aligned with structural changes on the landscape, including an emphasis on bobcat populations in the Black Hills. While there is evidence of fine-scale patterns of structure beyond $K = 4$, genetic clusters became geographically disjointed or were too small to meaningfully interpret. Consequently, for the purposes of harvest management $K = 4$ is likely to be feasible to implement, while still being cost-effective and capturing the most meaningful population genetic structure and genetic diversity of bobcats in the state (Allendorf et al. 2013).

We hypothesize that isolation by environment may be a considerable factor in the genetic structure we observed (Wang and Bradburd 2014). Wide-ranging carnivores, such as wolverines (*Gulo gulo*), coyotes (*Canis latrans*), and grey wolves (*Canis lupus*), also

exhibit genetic structure in areas that are relatively close, given their dispersal abilities, often due to ecological changes or anthropogenic disturbances (Cegelski et al. 2003, Sacks et al. 2004, Pilot et al. 2006). Bobcats throughout their range have exhibited both patterns of fine-scale population genetic structure as well as panmixia (Smith et al. 2020, Reed et al. 2017, Croteau et al. 2010, Reid 2006). In South Dakota, Reding (2011) found that bobcats clustered into a single population; our results expanded upon these previous findings with a larger sample size from South Dakota, the inclusion of East River samples, and a larger number of loci, which provided a more complete picture of population genetics in the state. The change in ecoregions on either side of the Missouri River and changes between the Black Hills and surrounding areas may have contributed to the genetic structure observed. The Black Hills region is the most disparate ecoregion in the state, and along the boundaries of the Black Hills was where we observed some of the highest levels of genetic differentiation for $K = 4$. Managers have observed telemetered bobcats moving out of the Black Hills (Chad Lehman, personal communication), but studies on bobcats and other terrestrial mammals have found that even when movement occurs between populations it does not necessarily lead to gene flow and the populations may maintain significantly different genetic structure (Koenig et al. 1996, Lee et al. 2012). Although we found that the Black Hills represented a unique population, and we hypothesized that this was due to isolation by environment, we did not have the fine-scale spatial data necessary to explicitly test this hypothesis.

We hypothesized the potential effect of linear features—specifically the major river and highway in the study area—through isolation by barrier (Vignieri 2005). Studies have found that rivers may not act as barriers to bobcats or their close relative the Canada lynx

(*L. canadensis*; Johnson et al. 2010; Feierabend & Kielland 2014). The Missouri River freezes in the winter, which coincides with juvenile dispersal and may further facilitate dispersal or extraterritorial movements when prey is scarce (Knick 1990). Consequently, the river may not be acting as a barrier, but instead may be acting as a natural corridor. This may be due to the vegetation that makes up the riparian zone on either side of the river being highly permeable to bobcats, given the availability of prey resources and the cover provided, as well as being preferable to the surrounding agricultural landscape, as other studies have suggested (Hilty & Merenlender 2004). While we did not observe patterns of population genetic structure that aligned closely with major roads or highways in South Dakota, Riley et al. (2006) found that roads may act as a barrier to gene flow for bobcats in some situations. Riley et al. (2006) investigated the influence of a freeway with heavy traffic in California, which they reported was used by >150,000 vehicles daily. In South Dakota, the heaviest traffic volumes were on I-29 and I-90, which received ~32,000 and ~21,000 vehicles daily on average, respectively (HPMS 2011). The relatively low traffic volume on South Dakota highways may be a contributing factor as to why we did not observe patterns of genetic structure that would be indicative of roads acting as barriers to bobcat gene flow.

Studies that use harvest data are susceptible to bias based on a number of factors, such as participation and reporting by the hunters, misidentification of sex, or if the harvest itself is biased towards a sex or age class (Williams et al. 2011, Schmidt et al. 2015). The use of harvest samples has been a cost effective and efficient means of sample collection for genetic analysis of carnivores such as wolverines and Eurasian lynx (*L. lynx*; Cegelski et al. 2003, Bagrade et al. 2006). In a similar study, samples from harvested bobcats and

county-level spatial data were used to detect genetic structure and inform harvest management in Oregon (Reding et al. 2013). Our study benefited from the CITES regulations that required bobcats to be checked by conservation officials, which eliminated the need for self-reporting by the hunters or trappers. Issues of field sex misidentification are not relevant given we conducted a molecular sex test for each sample. While harvest samples are non-random, a study in Wisconsin found that harvest of bobcats by hunters was more biased than harvest by trappers (Allen et al. 2018). During the extent of our study, from 2013–2019, over 75% of the bobcats were harvested by trappers (SDGFP 2019). It is also important to acknowledge that most of the land in South Dakota is privately owned and the use of harvest samples likely provided greater coverage of sampling across the study area than a random sampling could have achieved due to the lack of access.

Our inferences were limited by the resolution of our spatial data. Location data at the county level limited our ability to detect fine-scale impacts of roads and other features on structure. We were not able to interpret finer levels of structure, which limited our interpretation of results for analyses supporting $K = 10$ and $K = 12$. With more precise spatial data, we would have been able to conduct a landscape genetics focused project and evaluate specific landscape features on genetic structure of the population. Overall, we had a large sample size, but the sample size was disproportionate across counties, with counties west of the Missouri River, in general, providing more samples than the counties east of the Missouri River. This was, in part, due to the harvest regulations at the time of sampling, where the East River counties had a bag limit of only a single bobcat. In counties with very few samples, results may have been skewed by migrants that were

caught as they dispersed, and this could have influenced analyses that utilized spatial sampling information. Juvenile dispersal is variable but usually occurs late in their first year following separation from the mother prior to breeding and could align with the harvest season (Johnson et al. 2010). For example, our spatially explicit BAPS analysis supporting $K = 2$, there was a single county in the southeast portion of the state (Yankton) where more individuals assigned to the western cluster rather than the eastern cluster (Figure 3c). Only 3 of the collected samples were used from this county and at least 1 individual was assigned to a western cluster in each of our analyses. The influence of sample size on cluster characterization was more problematic at $K > 4$, where clusters with few individuals had F_{ST} and G''_{ST} values that were likely biased. In the case of the spatially implicit BAPS analysis, we were unable to calculate the *F*-statistics for a single cluster that had inadequate information due to a low number of samples assigned to the cluster. Large differences in the *F*-statistics between clusters with a small sample size are expected, as small sample sizes likely fail to adequately characterize the allele frequencies in each cluster (Hale et al. 2012). Even when the counties in the east had few samples, they clustered with counties just west of the Missouri River with more samples, strengthening support for these counties representing a single genetic cluster.

Both STRUCTURE and BAPS can correctly identify populations with low differentiation values (F_{ST} = 0.02–0.03, G''_{ST} = 0.20–0.28) when clustering individuals but may require higher levels of differentiation to correctly assign individuals to populations (Latch et al. 2006). Although, our values of F_{ST} and G''_{ST} at $K = 2$ and $K = 4$ were consistently lower than these thresholds, wild populations often have low *Fst* values <0.1, and this pattern has been observed in other studies evaluating F_{ST} in bobcats and other wild felids (such

as ocelots, *Leopardus pardalis*, jaguars, *Panthera onca*, and cougars; Holbrook et al. 2012, Figueiredo et al. 2015, Janečka et al. 2016, Wultsch et al. 2016). The spatially implicit Structure analysis we used was developed after the work of Latch et al. (2006) and improved STRUCTURE's ability to detect structure at lower levels of divergence without detecting structure that does not exist (Hubisz et al. 2009). STRUCTURE performed well with more complex population structure than evaluated by Latch et al. (2006), specifically in areas with contact zones (Evanno et al. 2005). Results from STRUCTURE interpreted using ΔK can be biased by a small sample size or a low number of markers (Evanno et al. 2005). Latch et al. (2006) based their conclusions on 500 genotypes simulated at 10 loci across 5 populations. Our sample included 878 genotypes of 10–17 loci (mean: 16.49, SD: 1.10) across our population clusters. Adequate characterization of allele frequencies for population genetic studies requires sampling of 25–30 individuals per population, or in cases of loci with high heterozygosity ($H_E \geq 0.70$), only 15–20 individuals (Hale et al. 2012). Mean H_E in our total sample was >0.70 in all but 2 loci (FCA098 and FCA205; Table 3). For each genetic cluster identified by analyses providing support for $K = 2$ or $K = 4$, H_E was >0.60 for all but two loci (i.e., FCA098 and FCA205; Tables 11–14). Although we would expect low levels of differentiation for a free-ranging, widely distributed, and mobile carnivore, such as bobcats, our large sample size and the number of loci used provided sufficient resolution to detect statistically significant evidence of population structure of bobcats in South Dakota.

MANAGEMENT IMPLICATIONS

The identification of hierarchical levels of genetic structure in the South Dakota bobcat population allows for finer scale management of these discrete populations. An eastern and western division into 2 genetic clusters was the most supported, but the support for finer levels of structure is important to take into consideration. The eastern genetic cluster identified at $K = 2$ and $K = 4$ was similar to current management units, although the East River unit would need to include counties that immediately west of the Missouri River and south of the Oahe Dam as well. Based on our findings, $K = 4$ genetic clusters is the resolution that may be the most beneficial from a management perspective and would separate management units into the East River, Black Hills, Northwestern and Southcentral areas. The 4 inferred genetic clusters were geographically congruent, captured the patterns seen at $K = 2$, and put specific emphasis on unique populations. This finer scale of management may assist in preventing the overharvest of populations that were not previously considered independent. Setting up management units to reflect the genetic populations in the state now would provide the framework for future changes in management, should the need arise for more specific harvest regulations for any of the identified populations. Bobcats are generally susceptible to harvest, and the precise management of these populations ensures their continued success in the state. Consequently, as more harvest occurs in the East River unit where we did not have access to samples, additional analyses will be necessary to identify structure within that unit as well.

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FIGURES

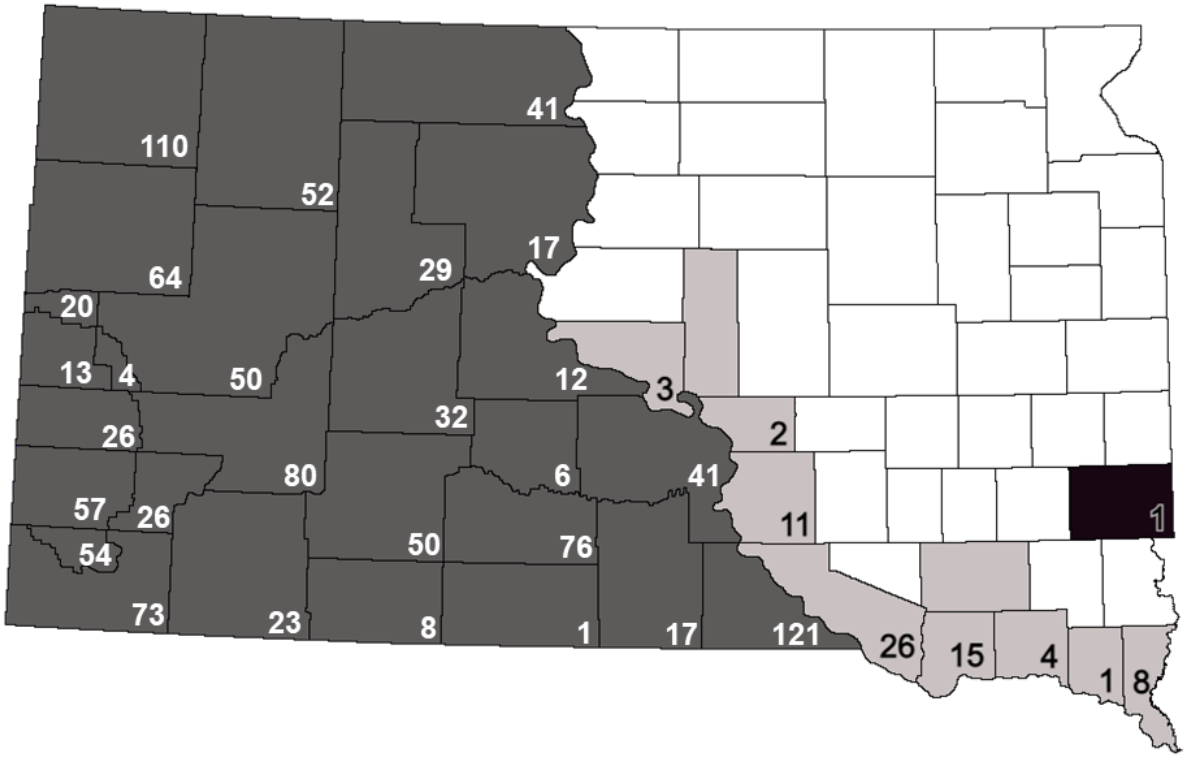

Figure 1. Counties (or subcounties) in South Dakota in which bobcat harvest was closed (white), counties in which bobcat harvest was open in gray (east of the Missouri River = light gray, west of the Missouri River = dark gray), and counties in which bobcat harvest was closed, but contributed samples (black) from December 2014–February 2019; the sample size contributed is in the bottom right corner of each county (or subcounty).

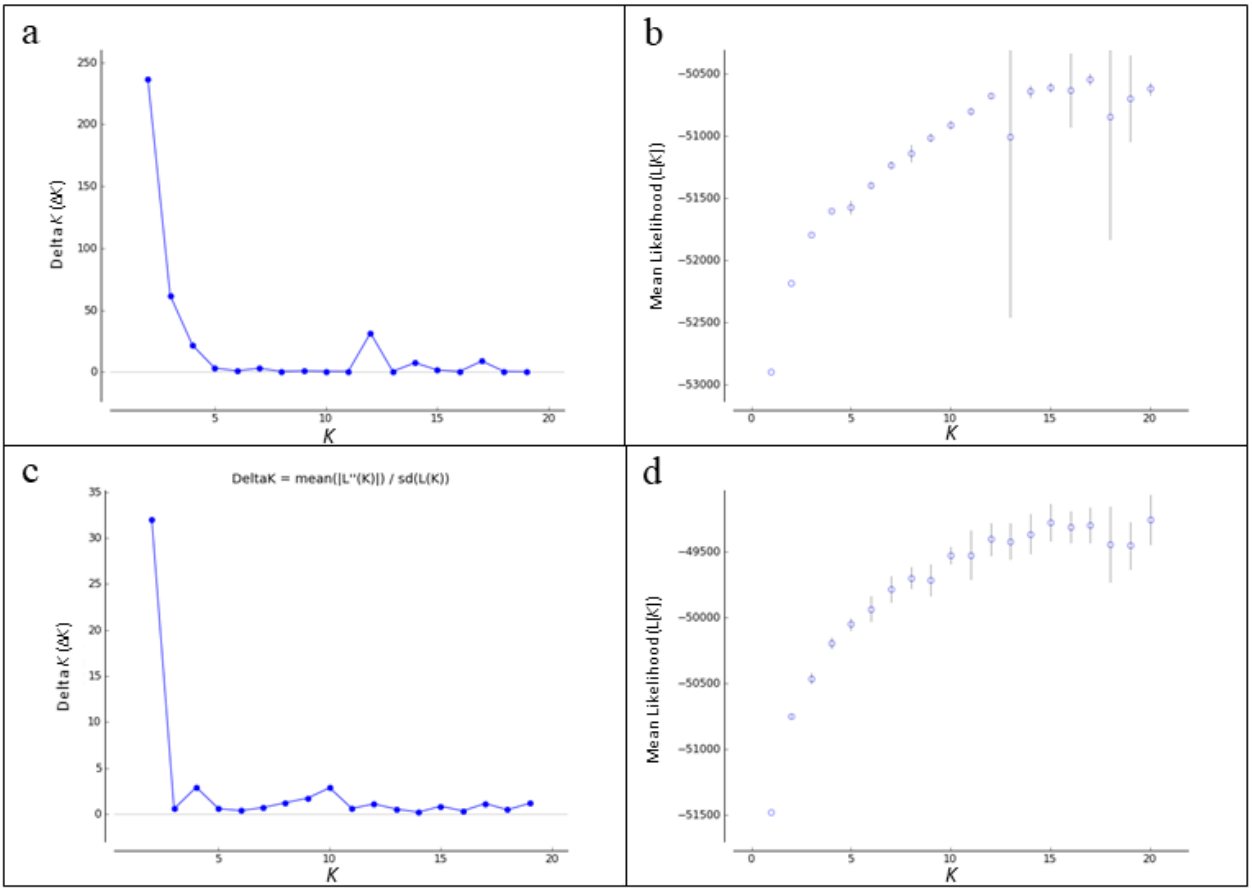

Figure 2. Plots of the mean log-likelihood (L[*K*]) ± 1 standard deviation and Delta *K*, the rate of change in L(*K*) for each subsequent *K* (Δ*K*), made in Structure Harvester for two STRUCTURE models: **a**) aspatial STRUCTURE model Δ*K*; **b**) aspatial STRUCTURE model L(*K*); **c**) spatially implicit STRUCTURE model Δ*K*; **d**) spatially implicit STRUCTURE model L(*K*).

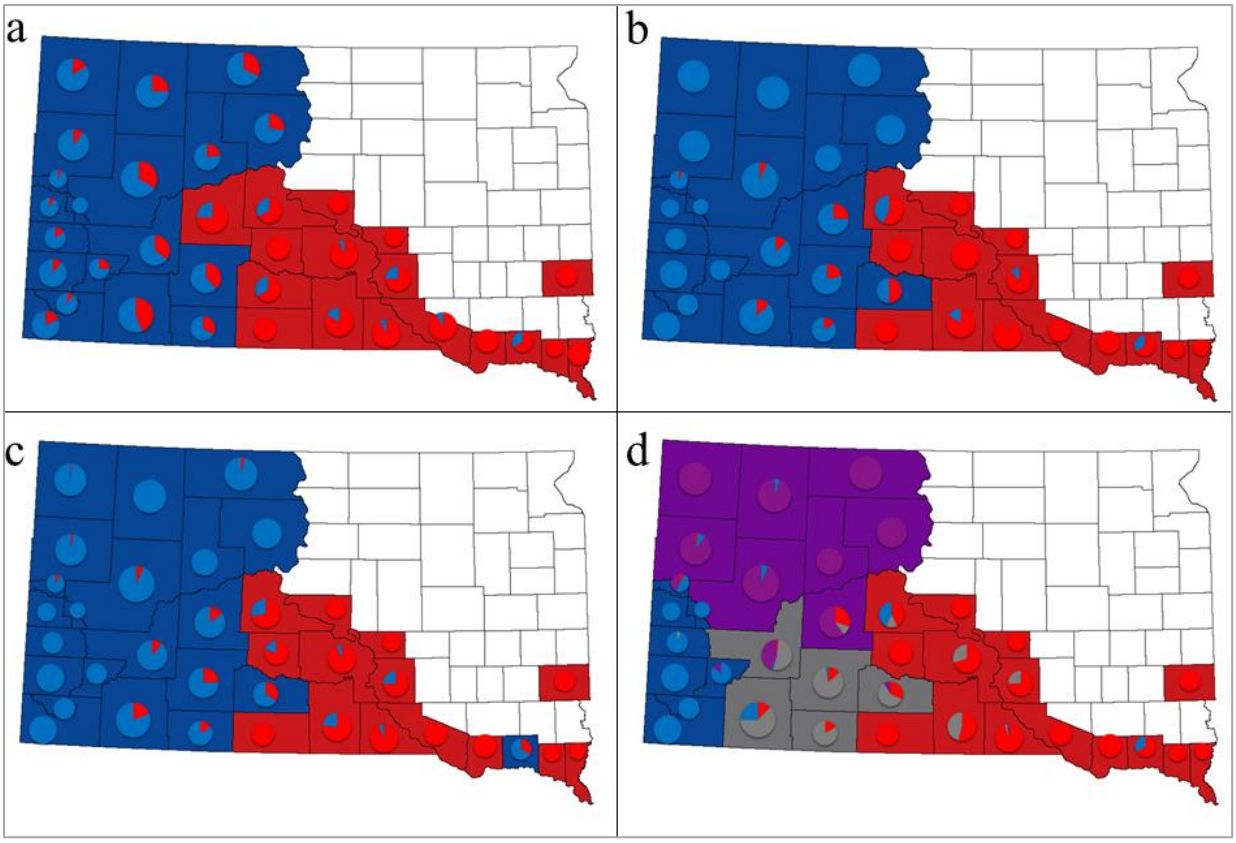

Figure 3. County-level visualizations of the Bayesian clustering algorithm results for harvested bobcats sampled in South Dakota from December 2014–February 2019, where each spatial group (county or subcounty) is assigned to the genetic cluster from which the highest proportion of individuals harvested in the county were assigned (as indicated by the pie chart). Analyses for $K = 2$ from **a**) aspatial STRUCTURE, **b**) spatially implicit STRUCTURE, and **c**) spatiality explicit BAPS identified eastern (red) and western (blue) clusters. Analysis for $K = 4$ from **d**) spatially implicit STRUCTURE analysis identified eastern (red), northwestern (purple), southcentral (gray), and Black Hills (blue) clusters.

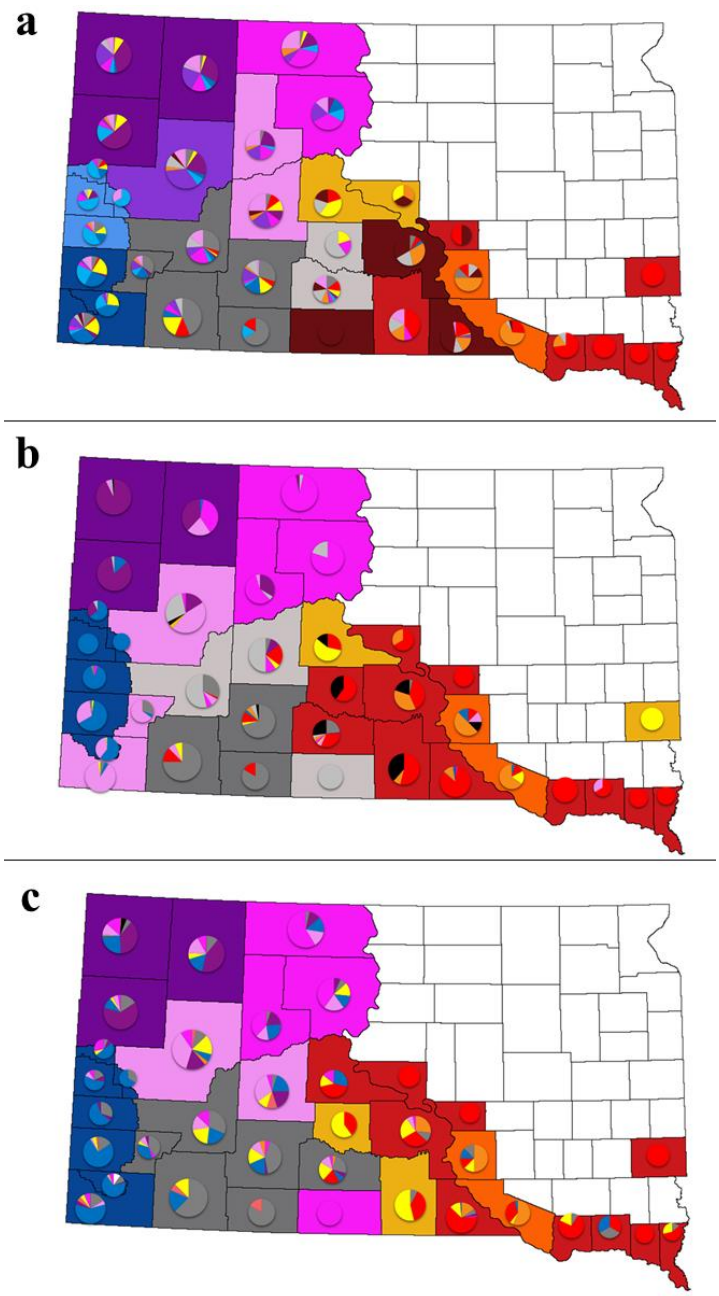

Figure 4. County-level visualizations of the Bayesian clustering algorithm results for harvested bobcats sampled in South Dakota from December 2014–February 2019, where each spatial group (county or county region) is assigned to the genetic cluster from which the highest proportion of individuals harvested in the county were assigned (as indicated by the pie chart). Analysis for $K = 12$ from **a**) aspatial STRUCTURE as well as analyses for $K = 10$ from **b**) spatially implicit STRUCTURE and **c**) spatiality implicit BAPS.

TABLES

Table 1. The volume (μL) and primer dye for the 17 nuclear DNA microsatellite loci that comprised the two multiplexes used for bobcat samples collected in South Dakota from December 2014–February 2019. All primers were used at a concentration of 10 μM.

Multiplex	Locus	Volume (μL)	Dye
Cougar Multiplex	F124[1]	0.03	PET
	FCA026[1]	0.04	PET
	FCA043[1]	0.03	NED
	FCA057[1]	0.05	VIC
	FCA082[1]	0.10	PET
	FCA090[1]	0.07	6-FAM
	FCA096[1]	0.08	6-FAM
	FCA098[1]	0.03	VIC
	FCA132[1]	0.08	NED
	FCA229[1]	0.04	6-FAM
Bobcat Multiplex	FCA008[1]	0.06	6-FAM
	F53[1]	0.20	NED
	FCA117[1]	0.25	6-FAM
	FCA205[1]	0.20	6-FAM
	FCA275[1]	0.09	VIC
	FCA391[2]	0.12	NED
	FCA741[1]	0.04	PET

Literature Reference: [1] Menotti-Raymond et al. 1999; [2] Mondol et al. 2012

Table 2. PCR thermal profiles for each multiplex used for bobcat samples collected in South Dakota from December 2014–February 2019.

Thermal profile	Step	Temperature (°C)	Duration
M1 Cougar	Initial Denaturation	95° C	0:15:00
	Touchdown	–	13 Cycles
	Denaturation	94° C	0:00:30
	Annealing	60° C – 0.8° C	0:01:30
	Extension	72° C	0:01:00
	Cycling	–	22 Cycles
	Denature	94° C	0:00:30
	Annealing	50° C	0:01:30
	Extension	72° C	0:01:00
	Final Extension	60° C	0:30:00
	Cooldown	04° C	0:10:00
M2 Bobcat	Initial Denaturation	95° C	0:15:00
	Touchdown	–	20 Cycles
	Denaturation	94° C	0:00:30
	Annealing	62° C – 0.6° C	0:01:30
	Extension	72° C	0:01:00
	Cycling	–	25 Cycles
	Denature	94° C	0:00:30
	Annealing	50° C	0:01:30
	Extension	72° C	0:01:00
	Final Extension	60° C	0:30:00
	Cooldown	04° C	0:10:00

Table 3. Genetic diversity metrics of bobcat samples collected in South Dakota from December 2014–February 2019. Each metric presented per locus for the total sample (n = 878) and a reduced set excluding samples with insufficient location data (n = 855): the number of alleles (A_N), allelic richness (A_R), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E), Weir and Cockerham's inbreeding coefficient (F_{IS}), as well as the standard error (*SE*) and *P*-value (*P)* for F_{IS}. *P*-values in bold indicate significant deviations from Hardy-Weinberg equilibrium after sequential Bonferroni corrections.

			Total finalized sample					**Finalized sample with spatial data**				
Locus	**A_N**	**A_R**	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***
F124	14	13.9	0.84	0.86	0.020	0.023	0.110	0.84	0.86	0.022	0.018	0.130
F53	16	15.8	0.81	0.82	0.011	0.002	**0.003**	0.81	0.82	0.011	0.007	0.014
FCA008	8	8.0	0.73	0.78	0.055	0.000	**0.000**	0.73	0.78	0.061	0.000	**0.000**
FCA026	15	14.8	0.82	0.84	0.022	0.001	**0.001**	0.82	0.84	0.021	0.002	**0.002**
FCA043	7	7.0	0.72	0.76	0.046	0.011	0.091	0.72	0.76	0.046	0.024	0.130
FCA057	13	12.8	0.81	0.85	0.053	0.000	**0.000**	0.81	0.85	0.053	0.003	**0.003**
FCA082	11	11.0	0.80	0.83	0.040	0.017	0.066	0.80	0.83	0.042	0.016	0.062
FCA090	7	7.0	0.70	0.79	0.117	0.000	**0.000**	0.70	0.79	0.121	0.000	**0.000**
FCA096	19	19.0	0.80	0.90	0.104	0.000	**0.000**	0.80	0.89	0.103	0.000	**0.000**
FCA098	11	11.0	0.52	0.77	0.324	0.000	**0.000**	0.52	0.77	0.324	0.000	**0.000**
FCA117	8	8.0	0.75	0.78	0.039	0.019	0.089	0.74	0.78	0.044	0.013	0.048
FCA132	8	8.0	0.77	0.81	0.047	0.023	0.407	0.77	0.81	0.047	0.026	0.520
FCA205	9	8.9	0.46	0.74	0.381	0.000	**0.000**	0.47	0.75	0.374	0.000	**0.000**
FCA229	12	11.9	0.80	0.81	0.020	0.000	**0.000**	0.79	0.81	0.022	0.000	**0.000**
FCA275	8	8.0	0.68	0.70	0.025	0.005	0.011	0.68	0.70	0.022	0.003	0.007
FCA391	7	7.0	0.71	0.72	0.019	0.014	0.068	0.70	0.72	0.027	0.009	0.057
FCA741	12	12.0	0.78	0.80	0.020	0.000	**0.000**	0.78	0.80	0.020	0.000	**0.000**
Mean	11	10.8	0.74	0.80	0.079	0.026	-	0.73	0.80	0.080	0.026	-

Table 4. Pairwise F_{ST} (below the diagonal) and G''_{ST} (above the diagonal) from GenAlEx between $K = 2$ inferred clusters from the aspatial STRUCTURE, spatially implicit STRUCTURE, and the spatially explicit BAPS analyses. All values were significant at $P < 0.001$.

	Aspatial STRUCTURE		**Spatially implicit STRUCTURE**		**Spatially explicit BAPS**	
K	**Eastern**	**Western**	**Eastern**	**Western**	**Eastern**	**Western**
Eastern		0.105		0.115		0.119
Western	0.012		0.013		0.014	

Table 5. Pairwise F_{ST} (below the diagonal) and G''_{ST} (above the diagonal) from GenAlEx between $K = 4$ inferred clusters from the spatially implicit STRUCTURE analysis. All values were significant at $P < 0.001$.

K	**Eastern**	**Northwestern**	**Southcentral**	**Black Hills**
Eastern		0.149	0.137	0.153
Northwestern	0.018		0.074	0.073
Southcentral	0.017	0.010		0.083
Black Hills	0.018	0.009	0.010	

Table 6. Pairwise F_{ST} (below the diagonal) and G''_{ST} (above the diagonal) from GenAlEx between K = 12 inferred clusters from the aspatial STRUCTURE analysis. All values were significant at $P < 0.001$.

K	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1		0.165	0.112	0.149	0.253	0.206	0.193	0.280	0.211	0.221	0.242	0.232
2	0.024		0.189	0.242	0.209	0.188	0.215	0.198	0.184	0.209	0.254	0.272
3	0.019	0.030		0.222	0.294	0.234	0.254	0.303	0.269	0.229	0.286	0.308
4	0.021	0.033	0.033		0.172	0.170	0.167	0.157	0.132	0.191	0.139	0.150
5	0.034	0.030	0.044	0.024		0.159	0.153	0.135	0.207	0.213	0.220	0.188
6	0.029	0.028	0.037	0.025	0.024		0.146	0.140	0.155	0.211	0.197	0.164
7	0.027	0.031	0.039	0.024	0.022	0.022		0.171	0.198	0.208	0.208	0.168
8	0.038	0.029	0.046	0.023	0.020	0.022	0.025		0.129	0.174	0.167	0.192
9	0.027	0.025	0.039	0.018	0.027	0.022	0.026	0.018		0.175	0.159	0.133
10	0.031	0.030	0.036	0.027	0.030	0.031	0.030	0.026	0.024		0.250	0.193
11	0.031	0.033	0.041	0.019	0.029	0.027	0.028	0.023	0.020	0.033		0.170
12	0.031	0.037	0.045	0.021	0.026	0.024	0.024	0.027	0.018	0.027	0.022	

Table 7. Pairwise F_{ST} (below the diagonal) and G''_{ST} (above the diagonal) from GenAlEx between K = 10 inferred clusters from the spatially implicit STRUCTURE analysis. All values were significant at $P < 0.001$.

K	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1		0.148	0.269	0.227	0.204	0.216	0.165	0.180	0.211	0.177
2	0.022		0.242	0.306	0.222	0.196	0.193	0.186	0.259	0.225
3	0.040	0.038		0.356	0.198	0.218	0.152	0.178	0.224	0.234
4	0.038	0.050	0.059		0.278	0.309	0.298	0.258	0.234	0.238
5	0.027	0.031	0.030	0.044		0.095	0.105	0.122	0.135	0.123
6	0.030	0.029	0.034	0.049	0.014		0.093	0.100	0.134	0.123
7	0.023	0.029	0.025	0.047	0.015	0.015		0.124	0.154	0.131
8	0.023	0.026	0.027	0.040	0.016	0.015	0.018		0.087	0.120
9	0.027	0.034	0.032	0.037	0.017	0.018	0.020	0.012		0.121
10	0.023	0.031	0.033	0.038	0.016	0.017	0.018	0.016	0.015	

Table 8. Pairwise F_{ST} (below the diagonal) and G''_{ST} (above the diagonal) from GenAlEx between K = 10 inferred clusters from the spatially implicit BAPS analysis. All values were significant at $P < 0.001$.

K	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1		0.162	0.156	0.208	nd	0.231	0.226	0.209	0.186	0.552
2	0.026		0.194	0.221	nd	0.208	0.218	0.214	0.246	0.614
3	0.022	0.029		0.162	nd	0.131	0.155	0.116	0.141	0.424
4	0.029	0.033	0.023		nd	0.099	0.124	0.143	0.118	0.506
5	nd	nd	nd	nd		nd	nd	nd	nd	nd
6	0.032	0.032	0.019	0.015	nd		0.134	0.107	0.114	0.482
7	0.032	0.033	0.022	0.018	nd	0.020		0.163	0.122	0.487
8	0.027	0.030	0.015	0.019	nd	0.015	0.021		0.076	0.350
9	0.023	0.033	0.018	0.015	nd	0.015	0.016	0.009		0.359
10	0.116	0.132	0.091	0.107	nd	0.103	0.103	0.076	0.076	

nd = No Data, indicating a cluster that had an insufficient number of samples to complete tests for F_{ST} or G''_{ST}

Table 9. Ancestry values (*q*) from the STRUCTURE admixture model for the aspatial analysis results at $K = 2$ and $K = 12$ for bobcats sampled from December 2014–February 2019. For each inferred cluster (*K*), the spatial area (i.e., description of the area in South Dakota where the cluster was geographically plotted), the number of samples assigned to each cluster (*n*), the number of samples with ancestry values >0.70 for their primary cluster ($q > 0.70$), the percent of samples out of the total assigned to each cluster that had ancestry values >0.70 for their primary cluster (% $q > 0.70$), and the percent of samples out of the total number of samples assigned to the cluster that had ancestry values <0.70 for their primary cluster (% $q \leq 0.70$) is reported.

K	**Spatial area**	***n***	***q* > 0.70**	**% *q* > 0.70**	**% *q* ≤ 0.70**
1	Eastern	362	221	61.0%	39.0%
2	Western	515	321	62.3%	37.7%
1	Eastern	74	20	27.0%	73.0%
2	Missouri River East	62	25	40.3%	59.7%
3	Missouri River West	64	29	45.3%	54.7%
4	Oahe Dam	74	13	17.6%	82.4%
5	Northwestern	88	25	28.4%	71.6%
6	Northwestern East	64	11	17.2%	82.8%
7	Black Hills Adjacent	79	15	19.0%	81.0%
8	Cheyenne River	72	13	18.1%	81.9%
9	Southcentral	81	22	27.2%	72.8%
10	Southcentral East	61	19	31.1%	68.9%
11	Black Hills South	82	25	30.5%	69.5%
12	Black Hills North	77	17	22.1%	77.9%

Table 10. Ancestry values (q) from the STRUCTURE admixture model for the spatially implicit analysis results at $K = 2$, $K = 4$, and $K = 10$ for bobcats sampled from December 2014–February 2019. For each inferred cluster (K), the spatial area (i.e., description of the area in South Dakota where the cluster was geographically plotted), the number of samples assigned to each cluster (n), the number of samples with ancestry values >0.70 for their primary cluster ($q > 0.70$), the percent of samples out of the total assigned to each cluster that had ancestry values >0.70 for their primary cluster (% $q > 0.70$), and the percent of samples out of the total number of samples assigned to the cluster that had ancestry values <0.70 for their primary cluster (% $q \leq 0.70$) is reported.

K	**Spatial area**	***n***	***q* > 0.70**	**% *q* > 0.70**	**% *q* ≤ 0.70**
1	Eastern	240	189	78.8%	21.3%
2	Western	615	535	87.0%	13.0%
1	Eastern	203	131	64.5%	35.5%
2	Northwestern	298	202	67.8%	32.2%
3	Southcentral	130	25	19.2%	80.8%
4	Black Hills	224	121	54.0%	46.0%
1.1	East	148	59	39.9%	60.1%
1.2	Missouri River East	45	20	44.4%	55.6%
1.3	Missouri River West	35	7	20.0%	80.0%
1.4	Oahe Dam	16	3	18.8%	81.3%
2.1	Northwestern	145	42	29.0%	71.0%
2.2	Northeast Northwestern	75	22	29.3%	70.7%
2.3	Southcentral	87	8	9.2%	90.8%
3.1	North Southcentral	62	5	8.1%	91.9%
4.1	Black Hills	119	14	11.8%	88.2%
4.2	Black Hills Adjacent	123	12	9.8%	90.2%

Table 11. Per locus and mean genetic diversity metrics for bobcat samples collected in South Dakota from December 2014–February 2019 for $K = 2$ (eastern and western) inferred genetic clusters from of the aspatial STRUCTURE analysis; metrics include allelic richness (A_R), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E), Weir and Cockerham's inbreeding coefficient (F_{IS} as well as the standard error (*SE*) and *P*-value (*P)* for F_{IS}. *P*-values in bold indicate significant deviations from Hardy-Weinberg equilibrium after sequential Bonferroni corrections.

	Eastern						Western					
Locus	**A_R**	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***	**A_R**	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***
F124	13.7	0.85	0.84	-0.020	0.007	0.016	12.2	0.83	0.87	0.040	0.019	0.194
F53	12.9	0.79	0.78	-0.018	0.036	0.236	15.0	0.82	0.83	0.012	0.012	0.044
FCA008	8.0	0.74	0.79	0.065	0.006	0.035	8.0	0.73	0.76	0.039	0.013	0.081
FCA026	13.0	0.82	0.82	0.008	0.017	0.081	14.2	0.82	0.82	-0.004	0.011	0.035
FCA043	6.0	0.72	0.77	0.065	0.013	0.180	7.0	0.72	0.72	0.003	0.017	0.423
FCA057	11.8	0.8	0.85	0.065	0.010	0.037	11.5	0.82	0.85	0.041	0.001	**0.001**
FCA082	10.0	0.79	0.82	0.031	0.013	0.065	10.0	0.81	0.82	0.019	0.027	0.443
FCA090	7.0	0.65	0.74	0.128	0.000	**0.000**	7.0	0.74	0.8	0.077	0.002	0.006
FCA096	19.0	0.78	0.87	0.100	0.000	**0.000**	14.8	0.82	0.89	0.087	0.000	**0.000**
FCA098	10.9	0.53	0.68	0.229	0.000	**0.000**	10.6	0.52	0.78	0.339	0.000	**0.000**
FCA117	7.0	0.71	0.77	0.068	0.005	0.018	6.9	0.77	0.79	0.015	0.013	0.130
FCA132	8.0	0.8	0.82	0.023	0.017	0.703	8.0	0.75	0.8	0.058	0.023	0.431
FCA205	8.0	0.44	0.7	0.365	0.000	**0.000**	8.5	0.47	0.76	0.381	0.000	**0.000**
FCA229	11.0	0.76	0.78	0.027	0.000	**0.000**	10.7	0.82	0.82	-0.007	0.000	**0.000**
FCA275	7.0	0.63	0.67	0.066	0.007	0.036	7.7	0.72	0.71	-0.005	0.027	0.272
FCA391	7.0	0.7	0.72	0.025	0.016	0.589	6.9	0.71	0.71	0.005	0.003	0.013
FCA741	12.0	0.76	0.78	0.029	0.001	**0.002**	11.6	0.8	0.81	0.009	0.000	**0.000**
Mean	10.1	0.72	0.78	0.074	0.023	-	10.0	0.75	0.80	0.065	0.028	-

Table 12. Per locus and mean genetic diversity metrics for bobcat samples collected in South Dakota from December 2014–February 2019 for $K = 2$ (eastern and western) inferred genetic clusters from of the spatially implicit STRUCTURE analysis; metrics include allelic richness (A_R), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E), Weir and Cockerham's inbreeding coefficient (F_{IS} as well as the standard error (*SE*) and *P*-value (*P)* for F_{IS}. *P*-values in bold indicate significant deviations from Hardy-Weinberg equilibrium after sequential Bonferroni corrections.

	Eastern						Western					
Locus	**A_R**	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***	**A_R**	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***
F124	12.8	0.85	0.84	-0.011	0.030	0.374	12.3	0.84	0.86	0.031	0.033	0.171
F53	11.7	0.77	0.77	-0.002	0.045	0.414	13.8	0.82	0.82	0.000	0.019	0.050
FCA008	8.0	0.76	0.8	0.046	0.015	0.109	8.0	0.72	0.76	0.059	0.000	**0.001**
FCA026	12.9	0.8	0.8	0.008	0.038	0.453	13.6	0.83	0.82	-0.008	0.003	**0.003**
FCA043	6.0	0.71	0.76	0.070	0.012	0.271	6.9	0.73	0.73	0.004	0.028	0.587
FCA057	10.9	0.81	0.87	0.066	0.016	0.081	10.3	0.81	0.84	0.042	0.016	0.091
FCA082	10.0	0.83	0.81	-0.020	0.021	0.765	10.2	0.79	0.82	0.047	0.036	0.246
FCA090	7.0	0.62	0.75	0.170	0.000	**0.000**	7.0	0.73	0.80	0.085	0.001	**0.001**
FCA096	19.0	0.8	0.88	0.082	0.032	0.072	14.2	0.80	0.89	0.101	0.000	**0.000**
FCA098	10.0	0.51	0.69	0.258	0.000	**0.000**	10.3	0.52	0.78	0.329	0.000	**0.000**
FCA117	7.0	0.71	0.77	0.077	0.011	0.129	6.6	0.76	0.78	0.026	0.026	0.154
FCA132	8.0	0.79	0.82	0.035	0.020	0.625	8.0	0.77	0.80	0.039	0.011	0.777
FCA205	7.9	0.48	0.71	0.324	0.000	**0.000**	8.1	0.46	0.75	0.387	0.000	**0.000**
FCA229	10.9	0.73	0.76	0.046	0.004	0.005	10.2	0.82	0.82	-0.003	0.005	0.009
FCA275	7.0	0.66	0.66	0.000	0.020	0.428	7.1	0.69	0.71	0.027	0.020	0.146
FCA391	7.0	0.71	0.73	0.034	0.013	0.833	6.8	0.70	0.71	0.015	0.009	0.035
FCA741	11.0	0.78	0.77	-0.015	0.002	**0.002**	11.0	0.79	0.81	0.026	0.000	**0.000**
Mean	9.8	0.72	0.78	0.069	0.023	-	9.7	0.74	0.79	0.071	0.027	-

Table 13. Per locus and mean genetic diversity metrics for bobcat samples collected in South Dakota from December 2014–February 2019 for $K = 2$ (eastern and western) inferred genetic clusters from of the spatially explicit BAPS analysis; metrics include allelic richness (A_R), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E), Weir and Cockerham's inbreeding coefficient (F_{IS} as well as the standard error (*SE*) and *P*-value (*P)* for F_{IS}. *P*-values in bold indicate significant deviations from Hardy-Weinberg equilibrium after sequential Bonferroni corrections.

	Eastern						Western					
Locus	**A_R**	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***	**A_R**	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***
F124	12.0	0.84	0.83	-0.005	0.018	0.093	12.4	0.84	0.86	0.027	0.016	0.131
F53	9.9	0.76	0.76	-0.003	0.032	0.364	13.6	0.82	0.82	0.001	0.002	**0.004**
FCA008	8.0	0.77	0.80	0.036	0.017	0.217	8.0	0.71	0.76	0.064	0.002	0.006
FCA026	12.0	0.79	0.80	0.006	0.034	0.272	13.5	0.83	0.83	-0.004	0.006	0.018
FCA043	6.0	0.71	0.76	0.074	0.018	0.201	6.8	0.73	0.73	0.005	0.023	0.652
FCA057	10.9	0.80	0.87	0.074	0.024	0.260	10.3	0.81	0.84	0.039	0.006	0.021
FCA082	10.0	0.81	0.81	-0.001	0.024	0.353	10.2	0.79	0.83	0.040	0.037	0.484
FCA090	6.0	0.62	0.75	0.169	0.000	**0.000**	7.0	0.73	0.80	0.088	0.000	**0.000**
FCA096	18.0	0.80	0.88	0.092	0.008	0.012	14.1	0.80	0.89	0.098	0.000	**0.000**
FCA098	10.0	0.51	0.68	0.256	0.000	**0.000**	10.2	0.52	0.78	0.327	0.000	**0.000**
FCA117	7.0	0.71	0.76	0.065	0.019	0.185	6.5	0.76	0.78	0.031	0.011	0.102
FCA132	8.0	0.81	0.82	0.019	0.010	0.867	8.0	0.76	0.80	0.045	0.021	0.590
FCA205	7.0	0.48	0.72	0.334	0.000	**0.000**	8.1	0.46	0.75	0.382	0.000	**0.000**
FCA229	11.0	0.71	0.74	0.046	0.000	**0.000**	10.3	0.82	0.82	-0.005	0.001	**0.001**
FCA275	6.0	0.67	0.66	-0.003	0.018	0.497	7.0	0.69	0.71	0.028	0.018	0.106
FCA391	6.9	0.71	0.73	0.032	0.022	0.489	6.8	0.70	0.71	0.015	0.006	0.027
FCA741	10.0	0.78	0.76	-0.020	0.001	**0.003**	11.3	0.78	0.81	0.027	0.000	**0.000**
Mean	9.3	0.72	0.77	0.069	0.024	-	9.7	0.74	0.80	0.071	0.027	-

Table 14. Per locus and mean genetic diversity metrics for bobcat samples collected in South Dakota from December 2014–February 2019 for $K = 4$ (eastern, northwestern, southcentral, and Black Hills) inferred genetic clusters from of the spatially implicit STRUCTURE analysis; metrics include allelic richness (A_R), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E), Weir and Cockerham's inbreeding coefficient (F_{IS} as well as the standard error (*SE*) and *P*-value (*P)* for F_{IS}. *P*-values in bold indicate significant deviations from Hardy-Weinberg equilibrium after sequential Bonferroni corrections.

	Eastern						Northwestern					
Locus	**A_R**	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***	**A_R**	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***
F124	11.9	0.83	0.83	0.000	0.040	0.348	11.6	0.85	0.86	0.013	0.011	0.056
F53	10.1	0.76	0.76	-0.005	0.031	0.278	11.6	0.84	0.80	-0.040	0.034	0.098
FCA008	7.6	0.74	0.79	0.066	0.009	0.074	7.7	0.68	0.73	0.072	0.023	0.546
FCA026	11.0	0.81	0.8	-0.009	0.018	0.087	11.7	0.81	0.81	0.005	0.004	0.008
FCA043	6.0	0.7	0.75	0.075	0.013	0.519	6.9	0.68	0.68	-0.003	0.012	0.129
FCA057	10.6	0.81	0.87	0.064	0.020	0.198	9.9	0.76	0.80	0.051	0.003	0.011
FCA082	9.7	0.81	0.8	-0.010	0.028	0.594	8.4	0.79	0.81	0.025	0.015	0.855
FCA090	6.0	0.62	0.73	0.160	0.000	**0.001**	7.0	0.70	0.79	0.119	0.001	**0.002**
FCA096	17.8	0.82	0.88	0.069	0.028	0.104	12.6	0.78	0.86	0.097	0.000	**0.000**
FCA098	9.9	0.53	0.66	0.209	0.000	**0.000**	9.4	0.52	0.79	0.336	0.000	**0.000**
FCA117	7.0	0.7	0.76	0.076	0.016	0.247	6.4	0.74	0.77	0.040	0.013	0.219
FCA132	8.0	0.8	0.82	0.030	0.016	0.787	8.0	0.72	0.76	0.054	0.022	0.698
FCA205	7.6	0.49	0.72	0.310	0.000	**0.000**	7.2	0.46	0.75	0.385	0.000	**0.000**
FCA229	10.5	0.71	0.76	0.063	0.000	**0.000**	8.8	0.85	0.81	-0.051	0.003	0.005
FCA275	6.2	0.69	0.67	-0.029	0.022	0.244	6.5	0.68	0.71	0.031	0.012	0.063
FCA391	6.5	0.69	0.72	0.034	0.020	0.508	5.7	0.69	0.68	-0.010	0.007	0.063
FCA741	10.2	0.78	0.75	-0.040	0.018	0.077	10.7	0.80	0.81	0.018	0.004	0.004
Mean	9.2	0.72	0.77	0.063	0.022	-	8.8	0.73	0.78	0.067	0.029	-

Table 14, Continued.

	Southcentral						Black Hills					
Locus	**A_R**	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***	**A_R**	**H_O**	**H_E**	***F_{IS}***	***SE***	***P***
F124	11.0	0.82	0.85	0.031	0.014	0.850	10.3	0.84	0.85	0.014	0.022	0.579
F53	10.0	0.81	0.80	-0.013	0.025	0.392	13.4	0.81	0.83	0.020	0.027	0.195
FCA008	8.0	0.81	0.80	-0.008	0.002	0.010	7.5	0.73	0.77	0.046	0.021	0.203
FCA026	12.0	0.84	0.83	-0.013	0.027	0.692	13.3	0.84	0.83	-0.010	0.012	0.034
FCA043	6.0	0.74	0.72	-0.026	0.006	0.103	6.0	0.78	0.76	-0.036	0.017	0.345
FCA057	10.9	0.79	0.82	0.039	0.024	0.183	10.0	0.88	0.88	-0.006	0.013	0.157
FCA082	9.0	0.79	0.82	0.041	0.018	0.692	9.9	0.80	0.83	0.033	0.006	0.967
FCA090	7.0	0.73	0.80	0.079	0.005	0.032	6.8	0.76	0.79	0.041	0.021	0.362
FCA096	13.0	0.82	0.88	0.067	0.023	0.201	13.8	0.80	0.90	0.105	0.000	**0.000**
FCA098	8.0	0.51	0.77	0.340	0.000	**0.000**	**10.5**	0.52	0.75	0.313	0.000	**0.000**
FCA117	6.0	0.70	0.76	0.078	0.005	0.019	6.5	0.81	0.78	-0.035	0.014	0.478
FCA132	8.0	0.82	0.83	0.009	0.014	0.249	8.0	0.80	0.81	0.015	0.015	0.815
FCA205	7.0	0.48	0.68	0.306	0.000	**0.000**	**7.5**	0.45	0.77	0.418	0.000	**0.000**
FCA229	10.9	0.81	0.80	-0.011	0.027	0.695	9.6	0.78	0.81	0.037	0.011	0.047
FCA275	6.8	0.65	0.70	0.069	0.021	0.273	6.5	0.70	0.71	0.010	0.024	0.632
FCA391	6.0	0.72	0.73	0.015	0.010	0.853	6.4	0.72	0.73	0.016	0.012	0.074
FCA741	10.9	0.78	0.81	0.033	0.004	0.012	10.3	0.77	0.78	0.022	0.023	0.592
Mean	8.9	0.74	0.79	0.061	0.025	-	9.2	0.75	0.80	0.059	0.029	-

Table 15. Per cluster (*K*) mean observed heterozygosity (H_O), unbiased expected heterozygosity (H_E), fixation index (F), and standard error (*SE*) for F of bobcat samples collected in South Dakota from December 2014–February 2019 from aspatial STRUCTURE analysis results for *K* = 12, spatially implicit STRUCTURE analysis results for *K* = 10, and spatially implicit BAPS results for *K* = 10.

K	Aspatial STRUCTURE				Spatially Implicit STRUCTURE				Spatially Implicit BAPS			
	H_O	**H_E**	**F**	***SE***	**H_O**	**H_E**	**F**	***SE***	**H_O**	**H_E**	**F**	***SE***
1	0.736	0.773	0.043	0.026	0.721	0.753	0.040	0.023	0.724	0.747	0.028	0.026
2	0.731	0.757	0.030	0.027	0.726	0.755	0.031	0.029	0.700	0.739	0.047	0.028
3	0.720	0.709	-0.024	0.020	0.703	0.743	0.040	0.030	0.723	0.771	0.060	0.030
4	0.751	0.774	0.021	0.026	0.754	0.752	-0.035	0.034	0.704	0.756	0.064	0.026
5	0.734	0.756	0.026	0.029	0.729	0.767	0.045	0.027	0.765	0.645	-0.350	0.058
6	0.716	0.750	0.040	0.043	0.729	0.757	0.030	0.033	0.720	0.758	0.049	0.043
7	0.711	0.755	0.053	0.028	0.725	0.771	0.053	0.032	0.723	0.760	0.042	0.036
8	0.734	0.752	0.020	0.028	0.755	0.790	0.041	0.025	0.755	0.794	0.047	0.024
9	0.767	0.790	0.025	0.026	0.751	0.790	0.047	0.029	0.761	0.799	0.046	0.028
10	0.699	0.758	0.073	0.035	0.745	0.786	0.051	0.031	0.802	0.646	-0.396	0.057
11	0.770	0.789	0.021	0.032	-	-	-	-	-	-	-	-
12	0.744	0.774	0.038	0.033	-	-	-	-	-	-	-	-

SUPPLEMENTAL MATERIAL

Table S1. All microsatellite loci screened for potential use in multiplexes for bobcat samples collected in South Dakota from December 2014–February 2019. All loci were tested with forward and reverse primers, initially at a volume of 0.09 µL and adjusted as needed in multiplex. Loci with multiple dyes listed were tested for each dye and the dye that was accepted is indicated in bold.

Locus	Dye	Multiplex
F124[1]	PET	M1
FCA026[1]	PET	M1
FCA043[1]	NED	M1
FCA057[1]	VIC	M1
FCA082[1]	PET	M1
FCA090[1]	FAM	M1
FCA096[1]	FAM	M1
FCA098[1]	**VIC** / 6-FAM	M1
FCA132[1]	NED	M1
FCA229[1]	FAM	M1
F53[1]	NED	M2
FCA008[1]	FAM	M2
FCA117[1]	FAM	M2
FCA205[1]	VIC / **6-FAM**	M2
FCA275[1]	VIC	M2
FCA391[2]	NED	M2
FCA741[1]	PET	M2
F41[1]	NED	Removed
F85[1]	VIC	Removed
F98[1]	FAM	Removed
FCA045[1]	PET	Removed
FCA075[1]	VIC	Removed
FCA124[1]	NED	Removed
FCA126[1]	PET	Removed
FCA166[1]	VIC	Removed
FCA225[1]	PET	Removed

Literature Reference: [1] Menotti-Raymond et al. 1999; [2] Mondol et al. 2012

Table S2. Per locus Weir and Cockerham's inbreeding coefficient (F_{IS}), as well as the standard error (*SE*) and *P*-value (*P)* for F_{IS}, of bobcat samples collected in South Dakota from December 2014–February 2019 from the aspatial STRUCTURE analysis results for K = 12. *P*-values in bold indicate significant deviations from Hardy-Weinberg equilibrium after Bonferroni corrections.

	Cluster 1			**Cluster 2**			**Cluster 3**			**Cluster 4**		
Locus	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*
F124	-0.015	0.014	0.126	-0.061	0.007	0.890	-0.057	0.015	0.608	0.006	0.019	0.185
F53	0.060	0.018	0.128	-0.023	0.023	0.448	0.015	0.051	0.342	0.003	0.008	0.081
FCA008	-0.113	0.016	0.354	0.099	0.006	0.073	0.034	0.011	0.829	0.077	0.017	0.295
FCA026	-0.045	0.035	0.658	-0.039	0.018	0.159	-0.028	0.027	0.366	-0.036	0.013	0.828
FCA043	-0.059	0.005	0.936	0.089	0.013	0.551	0.043	0.001	0.995	-0.077	0.020	0.707
FCA057	0.109	0.025	0.326	-0.011	0.017	0.795	0.003	0.019	0.396	-0.038	0.024	0.755
FCA082	-0.042	0.010	0.856	0.015	0.016	0.182	0.060	0.017	0.745	-0.021	0.014	0.744
FCA090	0.035	0.009	0.421	0.175	0.001	0.004	-0.044	0.015	0.540	0.053	0.013	0.460
FCA096	0.044	0.010	0.042	0.098	0.004	0.012	0.074	0.023	0.317	-0.022	0.003	0.012
FCA098	0.313	0.000	**0.000**	0.238	0.000	**0.000**	0.330	0.000	**0.000**	0.314	0.000	**0.000**
FCA117	-0.019	0.019	0.517	0.058	0.006	0.082	-0.020	0.015	0.386	-0.035	0.010	0.729
FCA132	0.013	0.015	0.307	0.003	0.001	0.995	-0.010	0.015	0.640	0.044	0.017	0.743
FCA205	0.234	0.005	0.021	0.299	0.000	**0.000**	0.247	0.002	0.006	0.289	0.000	**0.000**
FCA229	-0.055	0.017	0.163	0.012	0.022	0.319	-0.045	0.016	0.751	-0.137	0.003	0.022
FCA275	-0.009	0.013	0.311	-0.011	0.016	0.503	-0.089	0.011	0.189	-0.044	0.008	0.546
FCA391	0.023	0.013	0.156	0.007	0.016	0.681	-0.039	0.008	0.687	0.168	0.014	0.415
FCA741	0.054	0.016	0.512	-0.095	0.020	0.466	-0.005	0.006	0.022	-0.011	0.021	0.322

Table S2, Continued.

Locus	Cluster 5			Cluster 6			Cluster 7			Cluster 8		
	F**IS	***SE***	***P***	***F**IS***	***SE***	***P***	***F**IS***	***SE***	***P***	***F**IS***	***SE***	***P***
F124	-0.061	0.018	0.752	0.045	0.023	0.474	-0.001	0.030	0.256	0.025	0.017	0.063
F53	-0.032	0.047	0.516	-0.054	0.018	0.830	-0.035	0.023	0.637	-0.107	0.004	0.012
FCA008	0.035	0.014	0.325	0.000	0.014	0.362	0.092	0.009	0.069	0.012	0.019	0.223
FCA026	-0.038	0.015	0.062	-0.067	0.015	0.096	0.034	0.017	0.245	0.012	0.014	0.157
FCA043	-0.133	0.002	0.008	-0.006	0.011	0.658	-0.068	0.002	0.981	-0.043	0.014	0.211
FCA057	0.040	0.011	0.094	0.021	0.026	0.359	0.007	0.027	0.156	0.023	0.014	0.165
FCA082	0.056	0.029	0.459	0.011	0.008	0.942	-0.044	0.012	0.535	0.144	0.021	0.393
FCA090	0.067	0.013	0.693	-0.072	0.008	0.052	0.062	0.013	0.640	0.146	0.008	0.128
FCA096	-0.042	0.020	0.729	0.060	0.025	0.194	0.082	0.013	0.082	0.225	0.000	**0.000**
FCA098	0.282	0.000	**0.000**	0.307	0.000	**0.000**	0.343	0.000	**0.000**	0.245	0.000	**0.000**
FCA117	-0.062	0.009	0.163	-0.002	0.005	0.930	0.123	0.008	0.208	0.070	0.006	0.077
FCA132	-0.080	0.016	0.707	0.085	0.011	0.090	-0.079	0.021	0.844	-0.015	0.015	0.647
FCA205	0.388	0.000	**0.000**	0.415	0.000	**0.000**	0.521	0.000	**0.000**	0.304	0.000	**0.000**
FCA229	0.009	0.023	0.303	0.048	0.011	0.077	-0.037	0.007	0.877	-0.047	0.019	0.647
FCA275	0.050	0.015	0.363	0.018	0.014	0.760	0.145	0.002	0.007	0.070	0.011	0.252
FCA391	0.041	0.012	0.602	-0.158	0.010	0.103	-0.248	0.001	**0.003**	-0.080	0.009	0.799
FCA741	-0.061	0.019	0.689	0.115	0.022	0.299	-0.081	0.017	0.220	0.028	0.017	0.425

Table S2, Continued.

Locus	Cluster 9			Cluster 10			Cluster 11			Cluster 12		
	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*
F124	-0.017	0.025	0.429	0.056	0.014	0.189	-0.011	0.008	0.044	0.118	0.015	0.187
F53	0.030	0.021	0.437	-0.063	0.023	0.559	-0.160	0.021	0.273	-0.133	0.022	0.265
FCA008	0.074	0.011	0.338	0.023	0.011	0.709	-0.055	0.017	0.434	0.032	0.010	0.096
FCA026	-0.077	0.022	0.760	0.047	0.026	0.154	-0.008	0.019	0.189	-0.048	0.036	0.339
FCA043	0.041	0.009	0.686	-0.022	0.009	0.149	-0.005	0.006	0.907	-0.005	0.020	0.391
FCA057	0.046	0.013	0.502	0.046	0.024	0.357	-0.021	0.002	0.994	-0.039	0.012	0.906
FCA082	-0.063	0.014	0.317	-0.016	0.017	0.689	-0.074	0.006	0.966	-0.040	0.013	0.879
FCA090	0.208	0.000	**0.000**	0.198	0.004	0.016	-0.022	0.021	0.635	0.124	0.014	0.203
FCA096	0.099	0.029	0.681	0.185	0.005	0.012	-0.042	0.028	0.288	0.059	0.016	0.102
FCA098	0.111	0.009	0.108	0.381	0.000	**0.000**	-0.002	0.046	0.325	0.275	0.000	**0.000**
FCA117	0.010	0.005	0.745	0.022	0.008	0.807	-0.019	0.013	0.493	-0.045	0.008	0.370
FCA132	-0.041	0.013	0.646	-0.121	0.013	0.775	-0.015	0.003	0.982	0.057	0.015	0.364
FCA205	0.353	0.001	**0.001**	0.433	0.000	**0.000**	0.252	0.001	**0.001**	0.270	0.001	0.003
FCA229	0.050	0.015	0.808	0.080	0.035	0.549	-0.080	0.014	0.087	-0.093	0.008	0.064
FCA275	-0.005	0.006	0.643	-0.002	0.001	0.971	-0.001	0.001	0.988	-0.042	0.009	0.874
FCA391	-0.106	0.013	0.313	0.053	0.009	0.410	0.027	0.006	0.724	-0.007	0.005	0.070
FCA741	-0.052	0.014	0.088	0.088	0.008	0.079	-0.030	0.021	0.383	-0.021	0.025	0.514

Table S3. Per locus Weir and Cockerham's inbreeding coefficient (F_{IS}), as well as the standard error (*SE*) and *P*-value (*P)* for F_{IS}, of bobcat samples collected in South Dakota from December 2014–February 2019 from the spatially implicit STRUCTURE analysis results for $K = 10$. *P*-values in bold indicate significant deviations from Hardy-Weinberg equilibrium after Bonferroni corrections.

	Cluster 1			Cluster 2			Cluster 3			Cluster 4		
Locus	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*
F124	-0.034	0.012	0.130	0.008	0.028	0.600	0.145	0.001	0.005	0.052	0.025	0.577
F53	-0.051	0.028	0.178	0.039	0.020	0.181	-0.119	0.001	0.999	-0.091	0.009	0.274
FCA008	0.049	0.015	0.102	0.154	0.008	0.079	-0.037	0.013	0.716	0.003	0.015	0.656
FCA026	0.015	0.031	0.228	-0.153	0.022	0.710	0.006	0.001	0.996	0.006	0.012	0.391
FCA043	0.044	0.007	0.898	0.028	0.004	0.925	0.049	0.010	0.248	-0.145	0.007	0.926
FCA057	0.051	0.016	0.760	0.032	0.007	0.045	-0.076	0.022	0.460	-0.049	0.013	0.851
FCA082	-0.024	0.021	0.606	-0.125	0.008	0.202	-0.051	0.010	0.898	0.098	0.008	0.860
FCA090	0.093	0.011	0.163	0.252	0.002	0.016	0.205	0.006	0.066	-0.091	0.016	0.574
FCA096	0.067	0.030	0.084	0.098	0.008	0.887	0.137	0.020	0.093	0.137	0.028	0.314
FCA098	0.172	0.000	**0.000**	0.188	0.006	0.046	0.374	0.001	**0.001**	0.243	0.013	0.390
FCA117	0.106	0.000	**0.000**	-0.001	0.004	0.262	-0.033	0.010	0.439	-0.192	0.009	0.573
FCA132	0.016	0.008	0.926	-0.063	0.009	0.866	0.150	0.007	0.056	-0.040	0.009	0.703
FCA205	0.328	0.000	**0.000**	0.259	0.004	0.048	0.070	0.013	0.527	0.210	0.013	0.544
FCA229	0.008	0.003	0.008	0.103	0.013	0.115	-0.022	0.021	0.307	-0.114	0.022	0.602
FCA275	-0.040	0.019	0.576	0.001	0.010	0.268	0.093	0.011	0.588	0.233	0.005	0.087
FCA391	0.006	0.006	0.928	-0.093	0.005	0.380	-0.044	0.006	0.810	-0.080	0.001	0.989
FCA741	-0.062	0.014	0.091	-0.007	0.020	0.220	0.079	0.002	0.009	-0.196	0.008	0.692

Table S3, Continued.

Locus	Cluster 5			Cluster 6			Cluster 7			Cluster 8		
	***F*$_{IS}$**	***SE***	***P***	***F*$_{IS}$**	***SE***	***P***	***F*$_{IS}$**	***SE***	***P***	***F*$_{IS}$**	***SE***	***P***
F124	0.052	0.024	0.181	-0.048	0.020	0.861	0.018	0.020	0.461	-0.016	0.024	0.179
F53	-0.039	0.023	0.205	-0.057	0.016	0.652	0.013	0.016	0.094	-0.041	0.014	0.809
FCA008	0.099	0.010	0.090	0.032	0.008	0.935	-0.001	0.013	0.127	-0.021	0.013	0.532
FCA026	-0.014	0.020	0.736	0.015	0.017	0.098	-0.027	0.038	0.689	0.074	0.022	0.419
FCA043	-0.078	0.014	0.750	-0.077	0.012	0.638	-0.042	0.011	0.753	0.012	0.009	0.832
FCA057	0.011	0.017	0.172	0.103	0.022	0.205	0.120	0.005	0.035	-0.049	0.024	0.404
FCA082	0.001	0.021	0.317	-0.055	0.005	0.965	-0.007	0.014	0.765	0.043	0.016	0.520
FCA090	0.030	0.016	0.364	0.182	0.013	0.154	0.036	0.015	0.407	0.234	0.001	**0.002**
FCA096	0.110	0.000	**0.000**	0.009	0.020	0.127	-0.020	0.024	0.151	0.051	0.025	0.205
FCA098	0.278	0.000	**0.000**	0.327	0.000	**0.000**	0.313	0.000	**0.000**	0.324	0.001	**0.001**
FCA117	0.032	0.012	0.153	-0.029	0.009	0.424	0.071	0.013	0.616	0.050	0.007	0.781
FCA132	0.083	0.015	0.153	-0.004	0.013	0.797	0.038	0.008	0.139	-0.047	0.014	0.732
FCA205	0.333	0.000	**0.000**	0.367	0.000	**0.000**	0.289	0.001	0.003	0.407	0.000	**0.000**
FCA229	-0.073	0.008	0.038	-0.052	0.010	0.237	-0.048	0.017	0.310	0.025	0.018	0.198
FCA275	-0.035	0.017	0.330	0.047	0.010	0.145	0.045	0.015	0.226	0.049	0.015	0.486
FCA391	0.035	0.010	0.778	-0.090	0.006	0.756	-0.004	0.018	0.813	-0.014	0.007	0.147
FCA741	0.003	0.005	0.021	-0.042	0.004	0.013	0.001	0.022	0.192	-0.037	0.018	0.366

Table S3, Continued.

	Cluster 9			Cluster 10		
Locus	F_{IS}	***SE***	***P***	F_{IS}	***SE***	***P***
F124	0.007	0.025	0.470	-0.016	0.018	0.704
F53	-0.008	0.032	0.742	-0.013	0.020	0.079
FCA008	0.044	0.014	0.229	0.028	0.019	0.498
FCA026	-0.001	0.025	0.187	-0.086	0.029	0.398
FCA043	0.012	0.013	0.221	0.008	0.015	0.284
FCA057	0.007	0.011	0.108	0.011	0.013	0.854
FCA082	0.028	0.003	0.981	0.048	0.028	0.466
FCA090	0.014	0.023	0.554	0.038	0.012	0.648
FCA096	0.112	0.016	0.056	0.112	0.000	**0.000**
FCA098	0.251	0.000	**0.000**	0.336	0.000	**0.000**
FCA117	-0.082	0.011	0.522	0.023	0.011	0.555
FCA132	0.033	0.010	0.170	-0.057	0.016	0.347
FCA205	0.411	0.000	**0.000**	0.407	0.000	**0.000**
FCA229	0.038	0.027	0.168	-0.016	0.022	0.531
FCA275	-0.035	0.008	0.054	0.053	0.015	0.307
FCA391	-0.038	0.024	0.300	0.057	0.014	0.108
FCA741	0.087	0.022	0.186	-0.004	0.016	0.849

Table S4. Per locus Weir and Cockerham's inbreeding coefficient (F_{IS}), as well as the standard error (*SE*) and *P*-value (*P)* for F_{IS}, of bobcat samples collected in South Dakota from December 2014–February 2019 from the spatially implicit BAPS analysis results for *K* = 10. *P*-values in bold indicate significant deviations from Hardy-Weinberg equilibrium after Bonferroni corrections.

	Cluster 1			**Cluster 2**			**Cluster 3**			**Cluster 4**		
Locus	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*
F124	-0.062	0.024	0.263	0.071	0.015	0.396	0.073	0.011	0.023	0.075	0.027	0.553
F53	-0.060	0.024	0.654	0.103	0.020	0.408	-0.123	0.004	0.968	-0.030	0.011	0.768
FCA008	0.031	0.018	0.274	0.075	0.010	0.561	-0.031	0.003	0.979	0.122	0.013	0.300
FCA026	-0.003	0.014	0.119	-0.173	0.010	0.091	0.079	0.017	0.780	0.053	0.006	0.038
FCA043	0.079	0.011	0.215	0.058	0.004	0.927	0.016	0.008	0.870	-0.048	0.017	0.720
FCA057	0.027	0.021	0.322	0.164	0.004	0.062	-0.021	0.007	0.043	0.040	0.010	0.789
FCA082	-0.033	0.020	0.144	-0.079	0.009	0.678	-0.016	0.010	0.874	0.030	0.006	0.071
FCA090	0.046	0.011	0.470	0.228	0.004	0.014	0.223	0.001	**0.002**	0.110	0.000	**0.001**
FCA096	0.055	0.025	0.117	0.084	0.017	0.700	0.142	0.002	**0.003**	0.033	0.020	0.230
FCA098	0.226	0.002	0.004	0.237	0.003	0.112	0.273	0.000	**0.000**	0.319	0.000	**0.000**
FCA117	0.013	0.017	0.335	-0.022	0.004	0.841	0.111	0.009	0.182	0.035	0.012	0.146
FCA132	0.041	0.013	0.616	-0.058	0.009	0.884	-0.054	0.017	0.617	0.050	0.024	0.353
FCA205	0.351	0.000	**0.000**	0.191	0.019	0.246	0.354	0.000	**0.000**	0.285	0.000	**0.000**
FCA229	-0.027	0.007	0.039	0.104	0.008	0.497	0.038	0.018	0.285	-0.123	0.010	0.186
FCA275	-0.036	0.009	0.409	0.141	0.005	0.054	0.034	0.004	0.912	0.030	0.012	0.324
FCA391	-0.027	0.021	0.266	-0.059	0.006	0.739	0.019	0.005	0.056	0.143	0.010	0.324
FCA741	-0.072	0.017	0.596	-0.050	0.026	0.413	0.002	0.008	0.118	0.061	0.005	0.016

Table S4, Continued.

Locus	Cluster 5			Cluster 6			Cluster 7			Cluster 8		
	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*	F_{IS}	*SE*	*P*
F124	-0.412	0.000	1.000	-0.090	0.020	0.453	0.045	0.015	0.174	0.033	0.013	0.188
F53	-0.143	0.000	1.000	0.001	0.016	0.330	-0.163	0.018	0.181	0.038	0.010	0.039
FCA008	-0.412	0.000	1.000	-0.006	0.010	0.914	0.043	0.016	0.277	-0.022	0.020	0.247
FCA026	-0.200	0.000	1.000	0.055	0.014	0.386	-0.107	0.018	0.076	-0.013	0.031	0.327
FCA043	nd	nd	nd	-0.024	0.013	0.578	-0.031	0.003	0.964	-0.033	0.006	0.896
FCA057	-0.412	0.000	1.000	0.019	0.013	0.320	-0.100	0.026	0.397	0.095	0.030	0.312
FCA082	-0.286	0.000	1.000	-0.118	0.013	0.198	0.102	0.019	0.222	0.027	0.018	0.553
FCA090	0.000	0.004	0.658	0.139	0.002	0.009	0.073	0.007	0.164	0.104	0.007	0.069
FCA096	-0.500	0.000	1.000	0.134	0.006	0.046	0.213	0.000	**0.000**	-0.008	0.017	0.119
FCA098	0.500	0.005	0.142	0.358	0.000	**0.000**	0.332	0.000	**0.000**	0.311	0.000	**0.000**
FCA117	-0.500	0.000	1.000	-0.007	0.006	0.885	0.054	0.008	0.247	0.022	0.013	0.489
FCA132	-0.125	0.000	1.000	0.043	0.021	0.580	0.010	0.014	0.669	0.018	0.022	0.348
FCA205	-0.286	0.000	1.000	0.586	0.000	**0.000**	0.411	0.000	**0.000**	0.267	0.000	**0.000**
FCA229	-0.263	0.000	1.000	-0.002	0.019	0.337	-0.027	0.013	0.121	0.012	0.012	0.067
FCA275	-0.125	0.000	1.000	0.007	0.004	0.023	-0.026	0.010	0.671	-0.014	0.010	0.141
FCA391	-0.143	0.000	1.000	-0.009	0.010	0.352	-0.024	0.008	0.679	-0.014	0.010	0.163
FCA741	-0.333	0.000	1.000	-0.141	0.004	0.020	0.028	0.018	0.781	0.040	0.020	0.340

Table S4, Continued.

Locus	Cluster 9			Cluster 10		
	***F*$_{IS}$**	***SE***	***P***	***F*$_{IS}$**	***SE***	***P***
F124	-0.004	0.017	0.403	-0.250	0.000	1.000
F53	-0.040	0.032	0.385	-0.067	0.000	1.000
FCA008	0.089	0.002	0.013	-0.579	0.003	0.205
FCA026	-0.041	0.009	0.021	-0.579	0.004	0.204
FCA043	-0.019	0.014	0.648	-0.667	0.002	0.395
FCA057	0.043	0.010	0.077	-0.395	0.006	0.367
FCA082	0.016	0.013	0.928	-0.154	0.000	1.000
FCA090	0.009	0.012	0.105	-0.250	0.003	0.869
FCA096	0.085	0.006	0.011	-0.333	0.000	1.000
FCA098	0.288	0.000	**0.000**	-0.333	0.000	1.000
FCA117	-0.015	0.013	0.812	-0.290	0.000	1.000
FCA132	0.018	0.017	0.505	-0.395	0.007	0.353
FCA205	0.382	0.000	**0.000**	-0.111	0.000	1.000
FCA229	0.010	0.008	0.030	-0.250	0.000	1.000
FCA275	0.004	0.016	0.306	0.070	0.004	0.343
FCA391	-0.028	0.007	0.041	-0.600	0.002	0.429
FCA741	0.019	0.011	0.048	0.200	0.009	0.366

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